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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1984 INTRAMURAL RESEARCH PROJECTS

October 1, 1983 through September 30, 1984

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Z01 ES 21001-04 CTEB
Z01 ES 21003-04 STB
Z01 ES 21004-04 STB
Z01 ES 21009-03 STB
Z01 ES 21012-03 CGTB
Z01 ES 21013-03 CGTB
Z01 ES 21014-03 CGTB
Z01 ES 21016-03 CGTB
Z01 ES 21024-03 STB
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Z01 ES 21030-02 CTEB
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Z01 ES 21038-02 STB
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Z01 ES 21046-01 STB
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Z01 ES 90039-01 LBNT
Z01 ES 90040-01 LBNT
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OFFICE OF HEALTH HAZARD ASSESSMENT

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20003-11 OHHA

PERIOD COVERED: October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Preventive Surveillance of Environmental Chemicals for Toxic Potential

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Herbert S. Posner	Pharmacologist	OHHA NIEHS
Others:	William Jurgelski, Jr.	Medical Officer	OHHA NIEHS
	Warren T. Piver	Chemical Engineer	OHHA NIEHS
	Naomi Jean Bernheim	Microbiologist	OHHA NIEHS
	Errol Zeiger	Microbiologist	TRTP NIEHS
	Barry H. Margolis	Mathematical Statistician	BRAP NIEHS
	Jeanne Rimpo	Technical Staff Member	BRAP NIEHS

COOPERATING UNITS *(if any)*

Cellular Genetic and Toxicology Branch, TRTP/NIEHS
Statistics and Biomathematics Branch, BRAP/NIEHS

LAB/BRANCH: Office of the Director (OD)

SECTION: Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION: NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The relationship of chemical structure to mutagenicity in Salmonella is being examined using data provided by the National Toxicology Program. The first set of compounds considered are the nitro-compounds and their homologs which do not contain a nitro- group.

Examination of the structures of the nitro-compounds already tested and selected for testing aided in proposing 52 additional compounds for testing, about 60% of which were selected. Several treatments of the mutagenicity data have been developed to compare a broad spectrum of observed activities. Some of the findings can be summarized as follows. Primary nitroalkanes gave the lowest mutagenicity rates. The secondary nitroalkane, 2-nitropropane, however, yielded low but significant mutagenicity rates. Nitrobenzene was considered non-mutagenic. The trisubstituted, monocyclic aromatic compounds contained a greater number of compounds in the more mutagenic ranges than did the group of disubstituted derivatives. The sets of di- and trisubstituted compounds had the greatest numbers of representatives tested. With one exception, di- and trinitro-, tetrasubstituted monocyclic aromatic compounds were rather mutagenic. No mononitro-, tetrasubstituted derivatives have yet been tested. Some of the penta- and hexasubstituted compounds yielded the most variable data. As expected, some of the polycyclic aromatic and heterocyclic compounds were highly mutagenic. A homologous set of compounds that did not contain the nitro- group yielded mainly negative results or the compounds were weaker mutagens than their homologs.

A review of the hazards of methanol in individuals through nineteen years-of-age has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20007-07 OHHA

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification and Health Hazard Evaluation of Industrial and Food Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
Others:	Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
	Jean N. Bernheim	Microbiologist	OHHA	NIEHS
	Warren Piver	Chemical Engineer	OHHA	NIEHS
	David Pasquini	Air and Ind. Hyg. Engr.		RTI
	Larry Laird	Engineer		RTI

COOPERATING UNITS *(if any)*

Laboratory of Reproductive and Developmental Toxicology (LRDT/NIEHS)

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of two reviews on methanol toxicity (Methanol as a Pediatric Toxin) initiated in collaboration with Dr. H. Posner, has been completed and is being readied for submission to an appropriate journal.

An in-house review of chemical hazards in the microelectronics industry was completed.

Two new projects have been initiated:

1. A comparison of structure and function at the organ and system levels between the human and appropriate laboratory animals to permit selection of models for the normal and impaired human in toxicity evaluation of physical and chemical environmental agents, and

2. The development of guidelines that will permit clinicians to differentiate a disease with a chemical or physical etiology from those with an infectious or genetic causation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 20008-07 OHHA

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Marsupial Model in the Identification and Evaluation of Environmental Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William Jurgelski, Jr. Medical Officer OHHA NIEHS

COOPERATING UNITS *(if any)*

Office of Health Hazard Assessment

LAB/BRANCH

Office of the Director (OD)

SECTION

Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An invited review describing the use of the marsupial as a biomedical model is in preparation.

The invited review on the marsupial as a model in space biology is nearing completion.

Upon publication of the above papers, this project will be terminated. Future requests for information on the marsupial model will be considered as part of the project on Identification and Health Hazard Evaluation of Industrial and Food Chemicals. (201 ES 20007-07 OHHA).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 20011-04 OHHA

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comprehensive Evaluations of Biological Effects of Chemicals on Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Velimir B. Vouk	Visiting Scientist	OHHA	NIEHS
	Warren T. Piver	Chemical Enginer	OHHA	NIEHS
Others:	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
	Janet Guthrie	Microbiologist	OHHA	NIEHS
	Naomi Jean Bernheim	Microbiologist	OHHA	NIEHS

COOPERATING UNITS *(if any)*

Biometry and Risk Assessment Program (BRAP/NIEHS), Laboratory of Reproductive and Developmental Toxicoology (LRDT/NIEHS), WHO/IPCS Inter-Regional Research Unit (IRRU) at NIEHS and some 20 research institutions in the USA and other countries.

LAB/BRANCH

Office of the Director (OD)

SECTION

Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.3	2.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the year under review, the emphasis has been on methods for evaluating complex mixtures and for estimating chemical exposure and quantifying risks of chemical injury. Other activities included compiling monographs on chemicals selected for testing by the National Toxicology Program (NTP), evaluating exposure to and risks of ethylene dibromide (EDB), enumerating the hazards of sodium azide (the proposed inflator of air bags used as passive restraints in automobiles), and a compilation of all known work on thirty air pollutants regulated by the Environmental Protection Agency (EPA).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20014-01 OHHA

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
SGOMSEC Meeting on Method Development for Chemical Mixtures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Warren T. Piver Chemical Engineer OHHA NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.15	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreouced type. Do not exceed the space provided.)*

The Scientific Group on Methodologies and Safety Evaluation of Chemicals (SGOMSEC) held its 3rd Workshop in August 1983 in Guildford, U.K., to develop test methodologies for chemical mixtures. For the section of the Workshop on Evaluation of Mixtures, a manuscript was prepared on predicting concentrations of air pollutants.

The manuscript reviewed the meteorology and atmospheric chemistry of photochemical smog and the mathematical models used to predict ground level concentrations of pollutants. From this review, it was recommended that techniques be developed to measure the variability of velocity fields and dispersion coefficients for long-range transport of atmospheric pollutants and the measurement of transient free radicals and other very reactive chemicals that participate in the atmospheric reaction of photochemical smog to measure the variability. In addition, it was recommended that methods be developed to determine when simple models and single chemical models can be used as surrogates for predicting concentration profiles for chemical mixtures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20015-01 OHHA

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Estimation of Pollutant Concentrations in Air and Ground Waters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Warren T. Piver	Chemical Engineer	OHHA NIEHS
Others:	F. Thomas Lindstrom	Associate Professor Department of Mathematics	Oregon State Univ; Corvallis, Oregon
	Laila Moustafa		IPCS-IRRU WHO

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) A numerical procedure has been developed to describe the simultaneous transport of heat, chemical mass, and soil moisture in unsaturated soils. The deterministic model has been used to simulate transport of PCBs through different soils during light and moderate rain over 60 days. For unreactive chemicals, transport strongly depends on hydrodynamics and adsorptivities to different soil components.

(2) In conjunction with an Interregional Research Unit of the WHO/UNEP/ILO International Programme on Chemical Safety (IPCS), a workshop is being planned for May 1985. The workshop has three major objectives: (a) to review current knowledge on groundwater contamination; (b) to review current knowledge on the adverse effects of individual chemicals and chemical mixtures observed in ground water; and, (c) to provide guidance to Member States confronted with groundwater contamination and decontamination problems.

(3) For the 3rd Workshop of the Scientific Group on Methodologies and Safety Evaluation of Chemicals (SGOMSEC) to develop methods for testing chemical mixtures, a manuscript was prepared that reviewed and analyzed mathematical models used to predict ground level concentrations of atmospheric pollutants during photochemical smog incidents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 20016-01 OHHA

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Groundwater Quality Workshop

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Warren T. Piver Chemical Engineer OHHA NIEHS

Others: Laila Moustafa IPCS-IRRU WHO

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director (OD)

SECTION

Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

In conjunction with the IPCS of the WHO, a Workshop is being planned for May 1985. The Workshop is designed to accomplish three major purposes:

1) review basic knowledge on groundwater contamination from different sources, identify capabilities and limitations of available methods for measuring groundwater quality and predicting fate of mixtures of contaminants, and recommend neeeded research programs;

2) review basic knowledge on the toxic effects of individual chemicals and chemical mixtures observed in groundwater and recommend needed research programs; and

3) provide experience and guidance to Member States confronted with groundwater contamination and rehabilitation problems by analysis of actual contamination incidents.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25019-02 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physalaemin-like Peptide in Mammalian Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LBNT	NIEHS
Others:	W. E. Wilson	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biological Technician	LBNT	NIEHS
	O. Hernadez	Research Chemist	LEC	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina, Chapel Hill
University of Rome, Italy

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is continuing under Project Number: Z01 ES 90034-01, Title: Rabbit Stomach Peptide [Physalaemin-like Material (PLIM)] in Mammalian Tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50005-10 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Teruzo Konishi	Medical Officer (Res.)	LBNT	NIEHS
Others: J. Muratsuka	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurophysiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The perilymphatic perfusion with artificial perilymph containing sodium bromate (1mM) was employed in guinea pigs to study the ototoxity of bromate. Changes in the cochlear potentials and electrolyte concentrations in the endolymph were measured in both control and experimental perfusions. The perilymphatic application of bromate resulted in a gradual suppression of the endocochlear potential and sound evoked responses. The recovery of the cochlear potentials was not observed. A substantial decrease in potassium and chloride concentrations and increase in sodium concentration were observed in the endolymph. Our results indicated that the primary site of action of bromate was on the stria vascularis and in advanced stage of intoxication bromate might affect the hair cells, causing a further suppression of the hair cell responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50015-10 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Microwaves on Neural Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Donald I. McRee	Research Physicist	LBNT	NIEHS
Others: Clifford L. Mitchell	Supv. Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Non-Ionizing Radiation

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Frog sciatic nerves have been exposed to continuous wave (CW) and pulse microwave radiation. Rate of fatigue or loss of vitality (the ability of the nerve to continue firing under rapid stimulation) was increased in the nerve exposed to 2.45 GHz at a specific absorption rate of 10 mW/g. In order to determine if sine-wave modulated microwaves had an increasing effect on ionic transport as reported in the literature to occur in chick brain, frog sciatic nerves were exposed to 2.45-GHz microwaves sine-wave modulated at 8, 16, and 32 Hz. It was found that a 50 mW/g specific absorption rate was required to obtain a loss in vitality with this form of radiation. This result suggests that the nerve vitality is nonlinear with respect to microwave intensity. This type of nonlinear behavior would be expected if the neural membrane is acting as a diode-like detector of the microwave field. In order to study this membrane interaction and to obtain a basic understanding of the ionic transport and gating mechanisms in excitable membranes, the giant axons of the lobster ganglia are now being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50038-06 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of 2450 MHz Microwave Radiation on the Cardiovascular System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
Others: Donald I. McRee	Research Physicist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Duke University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Non-Ionizing Radiation

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to determine the influence of microwave radiation on cardiac tissue using in vitro and in vivo methologies. A method for exposing isolated rat atria to microwave radiation has been developed. The data suggest that 2.45 GHz CW microwave radiation of 2 or 10 mW/g has no overt effect on the rate of force of contraction of isolated atria. In addition, the response of atria to drugs was not influenced by microwave exposure. Specifically the dose response curve for isoproterenol and acetylcholine was not influenced by either 1, 10 or 100 mW/g exposure and the ability of propranolol and atropine to inhibit the isoproteronol and acetylcholine response of rat atria was not altered by these exposure levels. The influence of microwave radiation on the response of isolated atria to other cardiotonic drugs are being undertaken. Also, certain biochemical and physiological parameters, which are indicative of cardiac integrity, have been measured in unanesthetized rats during whole body ventral exposure to 2450 MHz CW microwaves. The data suggest microwave exposure of 10 mW/cm^2 for 6 hr has no effect on mean arterial blood pressure of colonic temperature. However, there was a microwave induced bradycardia which was exhibited after 30 min of microwave exposure at 10 mW/cm^2 and persisted throughout the remainer of the 6 hr exposure period. None of the biochemical or hematologic indices examined were influenced by this exposure level. Experiments on the effects of dorsal exposure and on the influence of elevated temperature on the response to microwave radiation have been initiated. No data are available for these experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50076-03 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Noise and Drugs on Water Control of the Cochlear Fluids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Teruzo Konishi	Medical Officer (Res.)	LBNT	NIEHS
Others:	Y. Muratsuka	Visiting Fellow	LBNT	NIEHS
	H. Ueda	Guest Worker	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurophysiology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to elucidate the mechanisms of water movement across the cochlear partition. Systemic injection of glycerin or deionized water was used to induce alterations in the osmolarity of the blood serum in guinea pigs. The cochlear potential including the endocochlear potential did not show marked suppression when the osmolarity of the cochlear fluids was elevated or lowered. Under normal conditions the osmolarity is significantly higher in the endolymph than perilymph. When the blood osmolarity was altered, the osmotic gradient across the cochlear partition decreased.

These studies are used as base line data to which water control in the cochlear fluids under pathologic conditions can be compared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90030-04 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Toxicants on Membrane-Related Neurochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen C. Bondy	Research Chemist	LBNT	NIEHS
Others:	T. Walsh	Staff Fellow	LBNT	NIEHS
	H. Tilson	Pharmacologist	LBNT	NIEHS
	C. Mitchell	Supv. Pharmacologist	LBNT	NIEHS
	S. Swartzwelder	Staff Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

U.S. Environmental Protection Agency
Duke University

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.9	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project emphasizes the nature of alterations to membranes of the nervous system by organometals for two purposes:

1. To delineate injury to discrete neuronal tracts and to account for observed behavioral changes in terms of damage to specific circuitry. This has been investigated by means of neurotransmitter and neuromodulator receptor analysis. Triethyl lead chloride reduces benzodiazepine binding in the rat hippocampus but not in frontal cortex or striatum. This depression is temporarily correlated with the analgesic effects of triethyl lead. Studies on neonatal rats exposed to triethyl or trimethyl tin also show a specific lowering of hippocampal benzodiazepine binding.

2. To assay for more general damage to cerebral membranes in organometal-treated rats and attempt to describe initial sites of damage caused by these compounds. Studies on membrane-transport systems of trimethyl tin treated animals show regionally distinct metabolic abnormalities. The ability to take up and concentrate amino acids from the plasma is specifically disrupted in the hippocampus while glucose uptake by this region is selectively and transiently elevated. This enhancement may reflect hyperactivity within the region which is ultimately responsible for the known regional morphological damage. Injury to membranes has been looked for by assay of lipid peroxide levels in brain areas and also levels of the protective enzymes superoxide dismutase and glutathione peroxidose. Elevated superoxide dismutase levels, preponderant in the hippocampus implied a reactive response to localized cellular injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90031-03 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of Neurophysiological Effects of Organometals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. L. Mitchell	Supv. Pharmacologist	LBNT	NIEHS
Others: S. Swartzwelder	Staff Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurophysiology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The organometals have numerous applications in industrial and occupational settings. The neurotoxicity of these agents, particularly organoleads and tins, is well known. However, their precise sites and mechanisms of action are poorly understood. The purpose of these studies is to characterize the neurophysiological effects of relevant organometals in an attempt to determine the site of action and aid in determining the mechanism of action of selected organometals. We have found that triethyl lead (TEL), but not trimethyl lead, triethyl tin or trimethyl tin markedly increases the sensitivity to pentylenetetrazol induced seizures. This increase in sensitivity is most pronounced in animals receiving multiple pentylenetetrazol injections. There is little, if any, change in sensitivity to a single dose of pentylenetetrazol. Thus, the most striking effect of TEL appears to be an acceleration of the pentylenetetrazol kindling process. This effect of TEL can be attenuated by anticholinergic agents (atropine and scopolamine). Assessment of the neurophysiological and neurochemical bases for pentylenetetrazol-induced kindling and the mechanisms whereby TEL exacerbates it should further our knowledge of excitatory phenomena of the nervous system and how these can be altered by neurotoxic substances.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90033-02 LBNT

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Milk Bombesin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator that Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LBNT	NIEHS
Others:	W. E. Wilson	Research Chemist	LBNT	NIEHS
	B. H. Irons	Biological Technician	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*:
University of Torino, Italy
University of Rome, Italy
University of Kyoto, Japan
University of North Carolina, Chapel Hill

LAB/BRANCH: Laboratory of Behavioral and Neurological Toxicology

SECTION: Peptide Neurochemistry

INSTITUTE AND LOCATION: NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new peptide containing immunoreactivity to bombesin was discovered in bovine milk, termed BMB. This peptide chemically differed from both the amphibian peptide and another mammalian bombesin-related peptide, GRP, isolated from porcine non-antral stomach tissue. Preliminary bioactivity studies further determined its differences from existing bombesin peptides, although it belongs to that family of peptides: BMB exhibited a bombesin-specific contraction of isolated rat uterus and guinea-pig large intestine and had a particularly evident effect on rhythmic movement. It was estimated to be 25-40% as active as bombesin and more potent than GRP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90034-01 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rabbit Stomach Peptide [Physalaemin-like Material (PLIM)] in Mammalian Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Wilson	Research Chemist	LBNT	NIEHS
Others:	L. H. Lazarus	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biological Technician	LBNT	NIEHS
	D. Harvan	Chemist	LMB	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*
University of Kyoto, Japan
University of North Carolina, Chapel Hill
University of Rome, Rome, Italy

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Peptide Neurochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
 - ☐ (a1) Minors
 - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term objectives of this research project are to identify and characterize this new peptide in neuronal tissue and to determine its physiological and pharmacological mode of action. This material was originally isolated as an immunoreactive substance that cross-reacted with a physalaemin-specific antiserum; mass spec date gave a mass ion of 866 and amino acid analysis indicated that it was an octapeptide. This peptide is not a tachykinin due to the absence of amino acids that typify that class of bioactive peptides. Preliminary data on its biological activities indicate an increase in tonus and phasic motility of guinea-pig ileal smooth muscle, with slower onset and relaxation than physalaemin. In rabbit large intestine, it appeared to inhibit tone and motility which was reversed by physalaemin. Thus, this peptide may prove to be a prototype of a new class of mammalian peptide hormones.

This was formally Project No. Z01 ES 25019-02.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90035-01 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Ornithine Decarboxylase in the Detection of Tissue Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Stephen C. Bondy	Research Chemist	LBNT	NIEHS
Others: J.-S. Hong	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to exploit the rapid changes in levels of ornithine decarboxylase (ODC) in tissues that respond to damage by regenerative or adaptive changes.

Chlordecone administration to rats, at levels causing tremor (40 mg/kg body weight) causes a 21-24-fold increase in levels of adrenal ODC. This is a rapidly occuring, reversible event of much greater magnitude than any other biochemical response to chlordecone hitherto reported. Other neurotoxic agents, such as triethyl lead chloride also cause a several-fold elevation in the adrenal level of this enzyme.

These data will be expanded so that the precise region of this effect will be determined together with a definition of those neuronal or endocrine inputs which are needed to allow this increase. A time course study allows the detection of those cerebral or peripheral areas which initially respond to the presence of various neurotoxicants.

The significance of this project is that sites of reaction to extremely low levels of toxicants may be detected by this means.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90036-01 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Animal Model of Organometal Neurotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	T. J. Walsh	Staff Fellow	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS
	R. L. McLamb	Technician	LBNT	NIEHS
	D. Schulz	Graduate Student	LBNT	NIEHS
	J. Chrobak	Graduate Student	LBNT	NIEHS
	S. Bondy	Research Chemist	LBNT	NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurobehavioral

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Organometal (OM) compounds are used for a variety of industrial applications and their trialkyl derivatives produce both neural and behavioral toxicity. The goals of this research are to characterize the time and dose-related neurotoxicity of OMs, examine the potential neurobiological substrates of these effects, and study their significance relative to the CNS. The behavior of animals exposed to OMs is similar to that of animals having limbic forebrain lesions. Pharmacological studies implicate catecholaminergic systems in some of the behavioral effects of triethyl lead. Subsequent studies have examined the effects of OMs on limbic system function. The limbic system modulates behavioral reactivity, emotionality and memory and appears to be very sensitive to many toxicants, including OMs. The effects of OMs have been compared to specific cytotoxicants such as AF64-A, 6-OHDA, and DSP-4. Studies to date indicate that the behavioral effects commonly associated with limbic damage appear to be related to a disruption of cholinergic function. These studies suggest that OMs and other neurotoxicants may be useful in the study of the neurobiological basis of some types of metabolic encephalopathies and degenerative disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90037-01 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toxicological Perturbations of Behavioral and Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	Charles F. Mactutus	Senior Staff Fellow	LBNT	NIEHS
	John S. Hong	Pharmacologist	LBNT	NIEHS
	Rosemarie M. Booze	Guest Worker	LBNT	NIEHS
	Jennifer Foshee	Graduate Student	LBNT	NIEHS
	Neachos Nearchou	Summer Student	LBNT	NIEHS

COOPERATING UNITS *(if any)*

The Johns Hopkins University
University of North Carolina at Chapel Hill

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of the central nervous system and its functional output, behavior, reflect a series of maturational events that are highly ordered and precisely timed; the consequences of early disruption can be acute and severe or more subtle and insidious.

Neonatal exposure to chlordecone, a prototypic organochlorine, produced tremor and increased reflex reactivity in preweaning rodents, effects similar to those observed in acute toxicity studies with adult animals. Sex-dependent body weight changes persisting into adulthood strongly implicated alterations(s) in the sexual differentiation of hypothalamic centers involved in feeding and weight regulation. Long-term alterations in brain catecholaminergic and serotonergic function were suggested by specific pharmacological challenges. Deficits in retention of learned responses dissociable from nonspecific alterations, such as hyperactivity, were also uncovered. Both short- (hypersecretion) and long-term (depression in basal levels) alterations in circulating and adrenal steroids indicated that chlordecone-induced changes in behavioral reactivity and the modulation of memory were produced, at least in part, by acting on the adrenal gland and the feedback regulation of the hypothalamic-pituitary-adrenal axis.

Neonatal exposure to triethyl lead (TEL), a representative organometal, was shown to permanently affect behavioral processes in a manner similar to that observed after damage to the septal-hippocampal system. Long-term behavioral alterations were independent of sensory modality and represented a hyperreactive response of the animal to the test environment. Preferential and permanent destruction of hippocampal pyramidal cell fields (regio inferior) were observed under light microscopy. Pharmacological probes suggested long-term alterations in cholinergic, but not dopaminergic, function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90038-01 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Model of Organochlorine Neurotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	C. F. Mactutus	Senior Staff Fellow	LBNT	NIEHS
	T. J. Walsh	Staff Fellow	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS
	D. Herr	Guest Worker	LBNT	NIEHS
	J. Gallus	Technician	LBNT	NIEHS
	J. Peterson	Technician	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Medical College of Virginia

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Organochlorine chemicals such as chlordecone, p,p´-DDT and lindane are widely used in developing countries and to a lesser extent in some industrialized countries. The purpose of this research is to characterize in an animal model the behavioral and neurological toxicity of chlordecone and study the mechanisms by which chlordecone and other organochlorine chemicals produce their neurotoxic effects. Our experiments have shown in rats that chlordecone-induced tremor can be differentiated from that of p,p´-DDT and permethrin by spectral analysis techniques. Lindane is convulsive, but not tremorigenic, while o,p´-DDT and mirex produce little neurotoxicity in rats. Pharmacological and neurochemical studies have indicated that the origin of the tremor is in the brain stem. While chlordecone and p,p´-DDT are similar in that they increase the turnover of serotonin and norepinephrine, they differ in that p,p´-DDT increases dopamine turnover and increases levels of excitatory amino acid transmitters; chlordecone has no effects on these measures. Other studies have shown that dilantin markedly attenuates the tremor produced by p,p´-DDT and permethrin and exacerbates the effects of chlordecone and lindane. These data support the interpretation that p,p´-DDT and permethrin act by holding the sodium channel open and indicate that chlordecone and lindane have different mechanisms of action. Future studies will concern the possible role of calcium in the neurotoxicity produced by these agents and the neuropharmacological basis for other neurobehavioral effects produced by these agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-90039-01 LBNT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modulation of Brain Opioid Peptides by Neuroleptics and Electroconvulsive Shock

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. S. Hong	Pharmacologist	LBNT	NIEHS
Others:	T. Kanamatsu	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neuropharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously demonstrated that repeated injections of haloperidol increase the concentrations of enkephalin in the striatum and nucleus accumbens, suggesting an intimate interrelationship between enkephalinergic and dopaminergic neurons. However, measurement of enkephalin level does not reflect the dynamic change of this peptide-containing neuron. Therefore, the first objective of this project was to develop methods to measure the turnover of brain enkephalin. We tried to measure the level of mRNA coding for enkephalin precursor (preproenkephalin A) as an index for the rate of biosynthesis by cell free translation or blot hybridization using cDNA clone for preproenkephalin A. The second objective was to examine the possibility whether enkephalin and other opioid peptides can be affected by another psychiatric treatment such as electroconvulsive shock (ECS). To study the modulation of opioid peptides by haloperidol and ECS, rats received daily administration of haloperidol (1 mg/kg, i.p. for 3 weeks) or ECS (150V, 1 sec. for 6-10 days) and were killed 24 h after the last administration. Repeated injections of haloperidol caused a two-fold increase in the striatal concentration of enkephalin. This increase was accompanied by a two-fold increase in the level of mRNA coding for the precursor of enkephalin. This suggests that haloperidol accelerates the turnover of enkephalin. Furthermore, this study demonstrates that long-term treatment with haloperidol affects the gene expression of the enkephalin system. This finding raises an important consideration that gene expression may be the ultimate site of action for antipsychotic drugs. Similar to haloperidol, repeated ECS also increased the brain concentration of enkephalin and level of mRNA coding for preproenkephalin A. This finding lends further credence that gene expression may be a common site of action for various psychiatric treatments. For future studies, we plan to use the newly developed cell free translation and blot hybridization methods to study the biosynthesis of enkephalin after haloperidol or ECS in greater detail. These studies should provide further information regarding the possible role of enkephalin in mediating the actions of haloperidol and ECS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-90040-01 LBNT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
On the Possible Mechanism of Chlordecone-Elicited Tremor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. S. Hong	Pharmacologist	LBNT	NIEHS
Others:	P. Chen	Expert	LBNT	NIEHS
	P. M. Hudson	Biological Lab Technician	LBNT	NIEHS
	J. Obie	Biological Lab Technician	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neuropharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlordecone (Kepone®) is a chlorinated hydrocarbon insecticide. One of the most severe and consistent neurological symptoms observed in exposed workers or laboratory animals was tremor. The purpose of this project was: a) To characterize the effects of chlordecone on the brain neurotransmitter systems such as biogenic amines, acetylcholine (Ach) and amino acid transmitters by measuring the rate of turnover of these neurotransmitters and to examine the neurochemical effects of chlordecone on hypothalamo-pituitary-adrenal function by determining the plasma levels of ACTH and corticosterone. b) To evaluate the contribution of each neurotransmitter system or hypothalamo-pituitary-adrenal-axis in the tremorigenesis of chlordecone. A single injection of chlordecone caused robust increases in the turnover rate of 5HT and NE, whereas the turnover of DA, Ach and GABA remained unaltered. The steady state concentrations of amino acid transmitters were also not affected. These results suggest the possible involvement of 5HT and NE in the tremorigenesis of chlordecone. This hypothesis was further supported by the finding that BC-105 (a serotonin receptor blocker) and phenoxybenzamine (an α_1 adrenergic receptor) significantly attenuated chlordecone-elicited tremor. Chlordecone also caused a great activation of pituitary-adernal function. A robust increase in the plasma levels of ACTH and corticosterone was found 2 hr after a single injection of chlordecone (75 mg/kg; i.p.). The hypertropic effect of chlordecone on the adernal and the pituitary was further confirmed by the morphological studies both at the light and the electron microscope levels. However, the possible link between the change in pituitary-adrenal function and chlordecone-elicited behavioral alterations such as tremor or hyperexcitability remains to be studied. For future studies, we plan to examine the possibility that chlordecone, like DDT, may exert its neurochemical or behavioral effects by acting on the soduim channel of the neuronal membrane.

LABORATORY OF GENETICS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60098-05 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mammalian Lactate Dehydrogenase Isozyme Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: Steven S.-L. Li Research Geneticist LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Study continued under Project Number Z01 ES 61032-01 LG entitled "Structure-function of mammalian lactate dehydrogenase isozymes".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60099-05 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organization-regulation of mammalian lactate dehydrogenase genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Hiroshi Tsujibo	Visiting Fellow	LG, NIEHS
	Kiyohito Yagi	Visiting Fellow	LG, NIEHS
	Kayoko Fukasawa	Visiting Fellow	LG, NIEHS

COOPERATING UNITS *(if any)*

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SECTION
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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

LDH-A cDNA clones from mouse and human have been isolated and sequenced. Mouse pMLA 73 was found to contain the 393bp of coding region and 496 bp 3' untranslated sequence including poly (A) tail. Four human cDNA clones consist of sequences of approximately 1,700bp, that is, the complete coding sequence (999bp), the 5' (97bp) and 3' (565bp) untranslated regions. Several LDH genomic clones have also been isolated and partially characterized from mouse and human DNA libraries. Mouse genomic clone M15 appears to contain a functional gene of at least 8Kb consisting of several exons and introns which have been sequenced. The nucleotide sequence of a human LDH-A pseudogene has been determined. 12.9% nucleotide differences were found between the pseudogene and LDH-A cDNA, and their significant implications on mammalian mutations and molecular evolution are observed. It is of interest to elucidate the genetic mechanism(s) underlying the tissue-specific expression of three LDH genes in human and other vertebrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60111-05 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Role of Gene 43 DNA Polymerase in Frameshift Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. S. Ripley	Senior Staff Fellow	LGM, NIEHS
Others:	J. G. de Boer	Visiting Fellow	LGM, NIEHS
	A. B. Clark	Biologist	LGM, NIEHS
	K. M. Price	Q	LGM, NIEHS
	D. M. Ferber	Q	LGM, NIEHS

COOPERATING UNITS *(if any)*
Institute of Molec. Biology — IAMP
Univ. of Oregon — NIH
Eugene, OR

LAB/BRANCH
Laboratory of Genetics

SECTION
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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2	1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our ongoing investigation of frameshift mutagenesis in the T4 rII system reveals the specific enhancement of particular pathways of spontaneous frameshift mutagenesis by different T4 DNA polymerases. Our studies now permit us to classify certain frameshift mechanisms by the genetic outcome (the nature of the DNA sequence change) and thus to examine the role of DNA polymerase defects exhibited by mutator and antimutator T4 DNA polymerases. For example, the mutator polymerase tsL98 produces a large increase in frameshift mutations occurring in runs of A:T base pairs (100 to 200-fold) while producing no increase in the addition of single base pairs not occurring in mononucleotide runs. Taken as a whole, our data strongly support the notion that frameshifts of these two types occur via different mechanisms.

Sequences of spontaneous frameshifts offer support for two novel mechanisms of frameshift mutation (Ripley, 1982; Ripley & Glickman, 1983; de Boer & Ripley, 1984). The first mechanism depends upon the correction of imperfect palindromic DNA sequences to more perfect palindromic DNA sequences. The second mechanism generates frameshifts by converting an imperfectly homologous sequence to a sequence that is perfectly homologous, but lies 256 base pairs down stream. This intragenic conversion event occurs at a substantial frequency in certain DNA polymerase mutant backgrounds examined. Sequences of proflavin-induced frameshift mutation in a wild type polymerase background demonstrate strong sequence specificity. Our initial results identify 14/16 mutants lie within a six base pair sequence. Not all of the mutations are identical (4 distinct genotypes have been observed). This demonstrates a direct sequence effect on occurence of frameshift mutations but may suggest the possibility of additional factors that define the specificity of these frameshift sites. Further studies in different mutant backgrounds and with other intercalating agents should permit a distinction between elements of site specificity dependent on the site of preferred mutagen binding from site specificity dependent upon site-specific DNA metabolic events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60113-05 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Mutagenesis in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. W. Glickman	Expert	LGM, NIEHS
	R. M. Schaaper	Visiting Fellow	LGM, NIEHS

COOPERATING UNITS *(if any)*

Dr. R. Fowler, Department of Biology
San Jose State University, San Jose, CA

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 60149-01 LG entitled "Molecular mechanisms of mutation: Mutational Specificity"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60130-03 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation of E. coli Mutants Defective in Repair of Alkylated DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. R. Volkert Senior Staff Fellow LGM, NIEHS

COOPERATING UNITS *(if any)*

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SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 60150-01 LG entitled "Gene Induction by Alkylation Treatments in E. coli"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60132-04 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction of recF143 and recA441 Mutations in DNA Repair and Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. R. Volkert Senior Staff Fellow LGM, NIEHS

COOPERATING UNITS *(if any)*

Alvin J. Clark and Linda J. Margossian
Department of Molecular Biology, Univ. of California
Berkeley, California 94720

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 60151-01 LG entitled "Role of the recF Gene in DNA Repair"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60136-03 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultraviolet Mutagenesis in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. W. Drake Head LGM, NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 60148-01 LG entitled "Error-Prone Repair in Bacteriophage T4"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60143-02 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mutagenesis in Mammalian Cells Using Cloned Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Zakour	Senior Staff Fellow	LGM, NIEHS
	B. W. Glickman	Expert	LGM, NIEHS
Others:	R. M. Schaaper	Visiting Fellow	LGM, NIEHS
	E. Drobetsky	Guest Worker	LGM, NIEHS

COOPERATING UNITS *(if any)*

Biology Department
York University
Downsview, Ontario, Canada

LAB/BRANCH

Laboratory of Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to examine at the molecular level the specificity of mutagenesis in mammalian cells. To do this, we are developing a system using cloned DNA sequences that can be shuttled into and out of the eucaryotic chromosome. The use of the cloned gene for thymidine kinase (TK) from Herpes Simplex Virus provides a defined target of known DNA sequence in which a broad range of mutagenic events, including nucleotide substitutions, frameshifts and small additions or deletions, can be examined. The specific aims of this project are to establish an experimental system in which this can be accomplished and to initiate an examination of the mutational specificity of a variety of potential environmental mutagens as well as the molecular nature of spontaneous mutagenesis in mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60145-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Mutational Specificity of Purified DNA Replication and Repair Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Senior Staff Fellow	LGM, NIEHS
Others:	J. C. Liu	Biologist	LGM, NIEHS
	J. L. Motto	Biologist	LGM, NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	0.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mutational specificity of highly purified and well characterized DNA replication and repair proteins is being examined in in vitro DNA synthesis reactions using biologically active viral DNA templates. Both a forward mutational assay capable of detecting a wide spectrum of errors (base substitutions, frameshifts, deletions, etc.) in M13 mp2 DNA, as well as highly specific reversion assays, are being employed. The work is presently focused on the accuracy of the proteins primarily responsible for the synthesis of new DNA, the DNA polymerases themselves. The accuracy of the three classes of eucaryotic DNA polymerases, established in the forward system, are very different; the mutation frequencies, per round of DNA synthesis in vitro are: polymerase -γ, 40 X 10^{-4}; polymerase -α, 80 to 200 X 10^{-4} and polymerase-β, 400 to 800 X 10^{-4}. DNA sequence analysis of over 1000 mutants indicates dramatic and informative differences in the kinds of mutations produced. For example, frameshift mutation frequencies are 300 X 10^{-4} for Polβ and <1.0 X 10^{-4} for Polγ. This correlates with the "processivity" of these enzymes. Comparably large differences in specific base substitution errors are also observed. These experiments are intended to provide detailed information on the parameters of protein-nucleic acid interactions which are important in determining accuracy. The studies are being expanded to employ prokaryotic DNA polymerases capable of proofreading, as well as additional "accessory" proteins from prokaryotic and eukaryotic systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60146-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mutagenic Consequences of Defined Lesions in DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Senior Staff Fellow	LGM, NIEHS
Others:	J. C. Liu	Biologist	LGM, NIEHS
	J. L. Motto	Biologist	LGM, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The exact mutagenic consequences of introduction of defined lesions into DNA are being examined in a forward mutational system using M13mp2 DNA and capable of detecting a wide spectrum of mutational events, both at and some distance away from the actual site of damage. The system has first been applied to depurination, the loss of a purine base from DNA. This is a frequent spontaneous lesion as well as a common intermediate in the repair of many other types of DNA damage. Depurination is highly mutagenic, as determined by transfection of depurinated M13mp2 DNA into SOS-induced compentent E. coli cells. DNA sequence analysis of 211 mutants demonstrates that most mutations are base substitutions reflecting insertion of dAMP opposite the non-coding abasic site and resulting in characteristic transversions. In order to determine the effects of a single abasic site placed at a defined position in the DNA, a general approach has been developed which should be applicable to many diverse types of premutagenic lesions. This procedure utilizes uracil containing DNA templates for standard in vitro reactions to incorporate the lesion into a covalently closed circular, and thus biologically active, molecule. The product of this reaction is then treated to yield, as the sole source of biological activity, complementary strand circles containing to lesion of interest at a single site and at essentially 100% frequency. In addition to its utility in studying lesions in DNA, the technique can be applied to standard site specific mutagenesis protocols to obtain very high efficiency even without selection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60147-01 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of SOS-Mutagenesis in Escherichia Coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. M. Schaaper Visiting Fellow LGM, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to study the molecular events that lead to mutation in the bacterium E. coli after induction of the SOS-response. The error-prone type of DNA replication that is presumed to be responsible for SOS-mutagenesis will be studied in an in vitro replication system. The accuracy with which crude extracts of E. coli cells copy normal or damaged single-stranded bacteriophage M13 DNA will be used as an indicator for in vitro SOS-expression. Characterization of the components involved is important for the study of SOS-mutagenesis and for the question of the regulation of mutation rates in general.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60148-01 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Error-Prone Repair in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head	LGM, NIEHS
Others:	F. W. Coleman	Senior Staff Fellow	LGM, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is an analysis of error-prone repair in bacteriophage T4. Most chemical and all radiation mutagenesis in T4 occurs via error-prone repair, and depends on the functions of the genes uvsW, uvsX, uvsY and virtually all genes required for DNA replication. We have recently obtained and characterized conditional (temperature-sensitive and amber) alleles of the X and Y genes. The ts mutants are of particular interest because they differentially affect three different traits of the mutants, namely suppression of a gene 49 (Holliday resolvase) defect, ultraviolet radiation sensitivity, and ultraviolet mutagenesis. We plan genetic tests of the effects of these mutations upon recombination (which also depends upon the WXY system), tests to ask whether recombination and mutagenesis are correlated, tests to determine which other genes of DNA metabolism are required for ultraviolet mutagenesis, tests of the sensitivity of mutants of the WXY system to photodynamic inactivation mediated by isopsoralen, tests to determine if the putative "uvsZ" gene is involved in error-prone repair, and screens for temperature-sensitive uvsW mutations. We plan to purify uvsX proteins from cells infected with ts uvsX mutants and to compare their biochemical properties with those of the wild-type protein, in order to determine which biochemical differences correspond to changes in survival versus mutagenesis after ultraviolet irradiation. Later, we plan to conduct the same kind of analysis of uvsY proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60149-01 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular mechanisms of mutation: mutational specificity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	B. W. Glickman	Expert	LGM, NIEHS
	R. M. Schaaper	Visiting Fellow	LGM, NIEHS
Others:	R. L. Dunn	Biologist	LGM, NIEHS
	D. L. Halderman	Biologist	LGM, NIEHS
	R. A. Zakour	Senior Staff Fellow	LGM, NIEHS
	B. N. Danforth	Bio. Lab. Tech.	LGM, NIEHS
	R. G. Fowler	Guest Worker	LGM, NIEHS

COOPERATING UNITS *(if any)*

Dr. R. Fowler, Department of Biology
San Jose State University, San Jose, CA

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to make reliable estimates of the risks presented by environmental mutagens an intimate understanding of the mutagenic lesions, and the DNA repair processes that moderate them, is essential. The achievement of such detailed insights requires both quantitative and qualitative data on mutation at the molecular level. This project involves the production of mutational spectra over a range of doses in different repair backgrounds. The data produced will provide a basis for the identification of mutational lesions, and improve our understanding of the role of DNA repair in mutation avoidance and mutation fixation. Determination of mutational specificity is carried out in the lacI gene of E. coli. Using genetic tools, amber and ochre mutations are identified, mapped and characterized by suppression with known suppressors. Since the DNA sequence is known we then know the base substitution required to produce the mutation. In addition a rapid cloning and sequencing technique has been developed allowing us to determine mutational spectra at the DNA sequence level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60150-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Induction by Alkylation Treatments in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. R. Volkert	Senior Staff Fellow	LGM, NIEHS
Others:	D. C. Nguyen	Chemist	LGM, NIEHS
	K. C. Beard	Q	LGM, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When E. coli cells are treated with sublethal levels of alkylating agents, they become more resistant to the mutagenic and lethal effects of subsequent high level treatments with alkylating agents. This increased resistance has been called the adaptive response and is the result of the induction of increased capacity to repair alkylation lesions in DNA. We have initiated a genetic study designed to learn what genes are induced by alkylation treatments, how they are regulated and what the functions of their products are. To date we have identified five genes or operons that are specifically induced by alkylation treatments by constructing fusions of the lac operon to promoters of genes induced by alkylation treatments. One of these alkylation inducible (aid) genes, or operons, (aidA) codes for the alkA gene product which is a glycosylase that removes methylated bases from DNA. aidD appears to be a fusion to the ada operon, which codes for two gene products, at least one of which is a regulatory protein controlling the adaptive response. The other three loci represent new genes involved in this process. Our studies show that treatments with levels of alkylating agents that elicit the adaptive response are suboptimal, and induce only a subset of the alkylation inducible genes. At optimal concentrations of alkylating agents, additional genes are induced. In addition, only four of the five loci are regulated by the ada gene. The fifth locus, aidC, is not only ada independent but is induced only by some methylating agents and not others. Thus the induction of genes by alkylation treatments is more complex than was originally revealed by studies of the adaptive response. We are now using these strains to learn how these aid genes are regulated and how their products function at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60151-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of the recF Gene in DNA Repair

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. R. Volkert	Sen. Staff Fellow	LGM, NIEHS
Others:	M. A. Hartke	Q	LGM, NIEHS

COOPERATING UNITS *(if any)*
Alvin J. Clark and Linda J. Margossian
Department of Molecular Biology, Univ. of California
Berkeley, California 94720

LAB/BRANCH
Laboratory of Genetics

SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The recF gene is required for two basic genetic processes, genetic recombination and DNA repair. The major question about recF function centers on whether recF functions in a regulatory capacity controlling these processes or whether it has an enzymatic function needed to carry out these reactions. Our studies of suppression of recF by several recA mutations suggests that the recF gene product functions primarily by modulating recA activity. It appears that two recF dependent changes in recA activity normally occur in response to DNA damage. The first change results in activation of the recA dependent proteolytic activity required for induction of the SOS pathways of DNA repair. The second change allows recA to carry out the RecF pathway of recombination and recombinational DNA repair. These two changes in recA activity are separable by mutation since the srfA mutation of the recA gene results in restoration of only RecF recombination without concomitant restoration of SOS induction in recF mutant strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61005-05 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and function of RNA Polymerase II in Drosophila melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Voelker	Research Geneticist	LG, NIEHS
Others:	L. L. Searles	Staff Fellow	LG, NIEHS
	S. S. Huang	Bio. Lab. Tech.	LG, NIEHS
	G. B. Wisely	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Arno Greenleaf, Department of Biochemistry
Duke University, Durham, North Carolina

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was initiated to genetically analyze the biosynthesis and function of the components of the RNA polymerase II transcription complex in Drosophila melanogaster. RNA polymerase II is a heteromultimer consisting of approximately ten different subunits, each of which is presumably specified by a different locus. The number of associated transcription factors (which are not structurally a part of RNA polymerase II) is unknown, but evidence for their existence has been found in other systems. To date only the genetic locus which specified α-amanitin-resistance to RNA polymerase II has been identified. That locus has not been cloned as recombinant DNA molecules and was found to encode the 215,000 dalton subunit. The genetic control of the biosynthesis of that subunit is being analyzed at the molecular level by analyzing a number of revertants of the P-element induced mutant that was used to clone the DNA sequences of the region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61011-05 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organization and Regulation of Gene Function in D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Research Geneticist	LG, NIEHS
Others:	Margaret W. Shen	Biologist	LG, NIEHS
	Patricia S. Davis	Chemist	LG, NIEHS
	Deborah A. Adams	Guest Worker	UNC, Chapel Hill

COOPERATING UNITS *(if any)*

Deborah A. Adams
University of North Carolina
Chapel Hill, N. C.

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Laboratory of Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study involves the genetical, cytological and molecular characterization of the white locus of Drosophila melanogaster. Comparisons of the normal gene structure with those of mutants that perturb the regulation of the locus are being made in an effort to understand the nature of the mutational changes and how they effect the function of the locus. The mutations currently under investigation result from the insertion/deletion of transposable elements near the 5' end of the gene.

Some of the mutants exhibit a mosaic of normal and mutant patches in the eye that reflect a clonal expression of the white gene while others are mosaic and show a nonclonal, nonautonomous expression. The two types of mutants are related to each other by descent and the objective is to determine the nature of the mutational changes and try to relate them to the way that regulation of the locus is modified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61018-04 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Sequence Variation in the Alcohol Dehydrogenase Gene Region of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles H. Langley	Research Geneticist	LG, NIEHS
	Charles F. Aquadro	Staff Fellow	LG, NIEHS
Others:	Susan F. Deese	Bio. Lab. Tech.	LG, NIEHS
	William F. Quattlebaum	Bio. Lab. Tech.	LG, NIEHS
	Brian Golding	Guest Worker	LG, NIEHS
	Douglas Billings	Biological Aid	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. C. Laurie-Ahlberg, Associate Professor of Genetics
North Carolina State University, Raleigh, North Carolina

LAB/BRANCH

Laboratory of Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Variation in the DNA restriction map in the Adh region (alcohol dehydrogenase locus) of chromosome II of Drosophila melanogaster from natural populations was examined. On average, 1 out of 50 nucleotides was observed polymorphic in the 12 kilobase region examined. In addition, we found insertions and deletions to be common, particularly in regions flanking the Adh transcript. All insertions of over 200 nucleotides share sequence homology with known transposable elements. The frequency distribution within Drosophila melanogaster and among related species suggests that such variants are deleterious mutants. Comparisons of Adh gene activity among inserted and noninserted sequences support this view for some variants. The effect of other flanking sequence variants may be on other adjacent genes and/or general chromatin structure. Evidence is found for preferential insertion of transposable elements into sequences implicated as being important in gene regulation (identified by their DNAse I hypersensitivity in intact chromatin). Two levels of Adh activity (high and low) commonly segregating in natural populations appear due to one or more nucleotide substitutions within the transcript that in some way alter standing levels of Adh mRNA (possibly by affecting mRNA stability or processing). These activity level substitutions appear to be distinct from, but in strong nonrandom association with, the two commonly segregating alleles of the protein (which differ by a single amino acid). It is clear that the target for mutations of significant effect is substantially larger than the coding sequence for the gene product.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61019-04 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Collaborative Protein Sequencing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*
Department of Diagnostic Immunology Research and Biochemistry, Roswell Park Memorial Institute, Buffalo, New York

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A glycoprotein exhibiting immunological and enzymatic activities of human prostatic acid phosphatase has been purified and amino-terminal sequences of both prostatic acid phosphatase and glycoprotein were found to be different.

A human prostate antigen was purified from both prostate gland and seminal plasma and its physico-chemical properties characterized.

Four ribonucleases have been purified to homogeneity and characterized from human seminal plasma. The seminal RNase III was found to be a prostate-specific enzyme, whereas others are similar to serum RNases.

Amino acid sequence of human dihydrofolate reductase has been determined, and the primary structure information is also helpful to understand the structure-function relationship of dihydrofolate reductase.

The primary structure information of protein is very important in elucidating the fundamental biological function. The collaborative research of protein sequencing will provide expertise so that fast and accurate information can be obtained for cloning and identification of eukaryotic genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61021-03 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Molecular Analysis of the cut Locus of D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph W. Jack	Senior Staff Fellow	LG, NIEHS
Other:	Willie Gibson	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in knowing how cells of a single organism can differentiate to form specific tissue types. We have chosen to address one aspect of the question by learning how one gene, the cut locus of Drosophila melanogaster, is expressed differently in different tissues of the fly.

We have now cloned DNA sequences representing the entire gene, which encompasses 150 kb or more of DNA. A large number of mutants have been analyzed. We find that the deletion of sequences in the leftmost part of the gene cause phenotypic effects primarily in the legs, while deletion of or insertions into sequences slightly to the right cause effects primarily in the wings. Mutations in a third region 70 - 80Kb away at the rightmost end of the gene prevent expression in both legs and wings and cause lethality of the flies when homozygous.

The availability of tissue specific mutants of a gene afford the opportunity to experiment to find out how the gene normally operates in tissue specific ways. We are currently studying the transcriptional activity of the cut locus and the DNA structure of normal and mutant alleles with the intention of finding out what causes the mutations and how the activity is altered.

We now know that many of the cut mutants are insertions of retrovirus-like sequences into the cut locus DNA, and we are interested in understanding the affect of these sequences on gene activity. Some of these mutations are suppressible and will be useful in determining how a mutation caused by a retrovirus-like sequence can be suppressed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61022-03 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Population Genetics of Transposable Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LG, NIEHS
Others:	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*
Dr. Norman Kaplan and Dr. Thomas Darden
Biometry and Risk Assessment Program

Dr. Brian Charlesworth
University of Sussex
England

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The evolutionary history of Drosophila transposable elements was studied. More than ten different families of transposable elements identified in D. melanogaster were found also in related Drosophila species. The single exception was the P element that was found only in stocks of D. melanogaster recently collected from wild populations. It appears that most transposable elements are stable components of the host genome. The single exception among those studies is the P element that may have recently invaded.

Theoretical studies of the extinction process of a transposable element family in a Mendelian host population were carried out. The impact of mutation within the transposable element to a defective copy was examined. Although values of various parameters can have large effects on the extinction time, it is generally true that mutation to defective elements accelerates the extinction process. This provides a scenario for the evolution of the P element in Drosophila melanogaster that rearranges to putative defective copies at a high rate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61023-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Drosophila Germ Cell Determination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. Boswell	Staff Fellow	LG, NIEHS
Others:	Steven A. Haneline	Biological Laboratory Technician	LG, NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is a fundamental concept in developmental biology that the fate of embryonic cells is regulated by morphogenetic determinants localized in the ooplasm. In Drosophila, heterotopic transplantation experiments have conclusively demonstrated that cytoplasmic factors localized to the posterior pole plasm of the oocyte and embryo are requisite for the formation of pole cells, the primordial germ cells. However, the molecular nature of these cytoplasmic factors, the mechanism of localization within the ooplasm, and their mode of action in development are unknown. The genetic and developmental analysis of maternal effect mutants that affect pole cell formation in Drosophila melanogaster are intended to allow one to elucidate the mechanism of determination and how the determined state is maintained throughout development.

A detailed genetic and developmental analysis of one such grandchildless mutant, tudor, (tud) has been undertaken. The properties of mutations of the recessive maternal effect gene tud indicate that the gene product of the tudor locus is required for the proper determination of germ cells in Drosophila melanogaster. Specifically, the germ plasm of six different alleles of tud has been analyzed at the ultrastructural level, and it is found that different alleles contain different amounts of assembled polar granule material (a cytoplasmic organelle classically thought to be the germ cell determinants). The ability or inability to form germ cells correlates directly to the amount of assembled polar granule material observed in the germ plasm. For example, one allele produces polar granules approximately 1/3 the size of wild type polar granules and this allele produces fertile progeny. On the otherhand, alleles that produces no apparent assembled polar granule material in the germ plasm produce no fertile progeny. Therefore, mutations at the tudor locus disrupt the normal assembly of the germ plasm resulting in the failure to localize the germ plasm determinants to the posterior pole.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61024-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Molecular Analysis of Suppressor-of-Sable Function in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Voelker	Research Geneticist	LG, NIEHS
Others:	D.-Y. Chang	Visiting Fellow	LG, NIEHS
	G. B. Wisely	Bio. Lab. Tech.	LG, NIEHS
	J. F. Sterling	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

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Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	2.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent findings in Drosophila have shown that 1) a significant proportion of spontaneous mutations are caused by insertions of mobile genetic elements, and 2) certain genetic suppressor systems are mediated through insertions of specific mobile elements. We are investigating the molecular mechanism of one such suppressor system: recessive mutations at the suppressor-of-sable [su(s)] locus suppress recessive mutations at the vermilion (v) locus that are caused by insertions of the mobile elements 412 and B104 (Project Number Z01 ES 61029-02 LG).

DNA sequences of su(s) have been cloned and are being characterized to determine the transcription orientation and the locations of the control and coding sequences. A primary goal of the work is to determine the protein product of su(s), a reduction or an absence of which effects suppression of several v alleles. Coding sequences from su(s) will be ligated into an expression vector to produce a fusion protein, against which antibodies can be produced. Antibodies against the su(s) portion of the fusion protein will be recovered and used as probes to identify the location and function of the su(s) protein product within the organism. The interaction of the su(s) protein with the v locus will be studied to determine how suppression is effected. By gaining an understanding of this phenomenon, we will learn if this type of suppression mechanism is an adaptive feature of Drosophila to deal with mutations caused by mobile element insertions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z0i ES 61025-02 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Sequence Variation in the Dopa Decarboxylase Region of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles F. Aquadro	Staff Fellow	LG, NIEHS
Others:	Susan F. Deese	Bio. Lab. Tech.	LG, NIEHS
	Charles H. Langley	Research Geneticist	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. C. Laurie-Ahlberg, Associate Professor of Genetics
North Carolina State University, Raleigh, North Carolina

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project has been to examine the patterns and levels of naturally occurring DNA sequence variation in and around a dense cluster of lethal-mutable genes in Drosophila melanogaster. Of particular interest was the affect of sequence alternations within and flanking the gene encoding the enzyme dopa decarboxylase (Ddc). No clear patterns between sequence variants and Ddc expression were observed, despite two-fold variation in DDC enzyme activity among the 46 lines examined. Located adjacent to the 30 - 40 kilobases of DNA sequence containing the dense cluster of eight lethal-mutable genes (including Ddc) is an approximately 40 kilobase region in which, at most, one gene appears to be located. We were interested in levels of sequence variation, particularly deletions and insertions (including transposable elements), in this latter region. If mutations occur randomly throughout the genome, yet persist for any length of time only in regions where they produced little or no deleterious effect, we should see less variation in the dense cluster of genes than in the adjacent 40 kb region. Examination of DNA sequence variation by restriction mapping in this 80 kilobase region has revealed the opposite pattern. Virtually no variation was observed in the genetically sparse region while numerous insertions/deletions in the region containing the dense cluster of genes. While we cannot rule out the selective maintenance of variation in the gene cluster, these results raise the possibility of increased insertion/deletion mutational activity in transcriptionally active regions although unknown constraints may exist in the nonvariable region. We are testing these hypotheses in several other regions of the Drosophila genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61026-02 LG

PERIOD COVERED
October 1, 1983 to December 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mammalian Mitochondrial DNA Variation and Evolution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles F. Aquadro	Staff Fellow	LG, NIEHS
Others:	Norman Kaplan	Mathematician	BRAP, NIEHS
	Kenneth J. Risko	Mathematician	BRAP, NIEHS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project was to determine the major features of mammalian mitochondrial DNA sequence variation and evolution as it reflects on the underlying mechanisms of mutation. Human mitochondrial DNA sequence comparisons were made, resulting in the detection of high frequencies of multiple (repeated) nucleotide substitutions and insertions/deletions. Two substitution biases were apparent, one favoring transitions by a factor of 32:1 over transversions and the other favoring a high rate of turnover of purines relative to pyrimidines on the heavy strand of mtDNA. Their occurrence in coding and non-coding regions as well as ribosomal RNA and transfer RNA genes suggests that these phenomena may result from biases in the mutational pathways since it is unlikely that similar selective constraints would exist in these functionally very different regions. We have also modeled the dynamics of the substitution process in mammalian mtDNA. We have studied the temporal behavior of several quantities and compared the model's predictions with estimates obtained from recent mtDNA sequence data for an increasingly divergent series of primates, the mouse and the cow. Our results are consistent with the hypothesis that the decrease in the proportion of transitions observed as divergence increases is a consequence of the highly biased substitution process. In addition, our results support the hypothesis that while a portion of the mtDNA molecule evolves at an extremely rapid rate, a significant portion of the molecule is under strong selective constraints related to conservation of protein sequence and transfer RNA secondary structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61027-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transmission of Cryptic Mutations in Destabilized X Chromosomes of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Research Geneticist	LG, NIEHS
	J. W. Jack	Senior Staff Fellow	LG, NIEHS

COOPERATING UNITS *(if any)*
Dr. Johng K. Lim, Professor of Biology
University of Wisconsin, Eau Claire

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A stable Drosophila X chromosome can be destabilized by association for only one generation with the unstable X chromosome (Uc) or its derivatives. Occasionally, male flies carrying the destabilized X chromosome transmit a mutant allele not apparent in their phenotype (cryptic mutation). We are interested in learning about the origin and the underlying molecular mechanisms for transmission of cryptic mutations.

In our system, two X-linked loci exhibit a high frequency of generation and transmission of cryptic mutation. These are cut wings (ct) and forked bristles (f) loci. In a particular cross, for example, more than 2% of males carrying the destabilized X chromosomes produce at least one sperm with cryptic ct. Our data show that a transposable element called gypsy is involved in transmission of the mutations. A number of separate lines of evidence indicate that the ordinary meiotic recombination process is not involved in generating the cryptic mutations.

Suppressible insertion mutations, transposon-mediated instability, heteroduplex structure of cryptic mutant loci, or involvement of extrachromosomal replicating elements are some of the models we have been testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61028-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mutations Affecting the Expression of an RNA Polymerase II Locus in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lillie L. Searles	Staff Fellow	LG, NIEHS
Others:	Robert A. Voelker	Research Geneticist	LG, NIEHS
	Mary L. Tate	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to characterize mutational events associated with insertion and excision of the transposon P element at the RNA polymerase II locus, $RpII^{215}$, of Drosophila. Several P element insertion mutations which affect the expression of this gene have been analyzed at the molecular level. The insertions are clustered in two small subintervals of the locus, one region within coding sequences and the other region near the 5' end of the gene. Detailed analyses have focused on two of these insertions, D50 and W42, both of which lie near the 5' end of $RpII^{215}$ and reversions of D50 which restore variable amounts of activity to the gene.

Wild type DNA in the 5' region of the $RpII^{215}$ gene has been sequenced. Within this region the sites of insertion of mutants D50 and W42 have been localized by sequencing. Although both insertions lie within the same region, the precise site of W42 insertion is 80 base pairs upstream of the D50 insertion site.

DNAs from four D50 revertants have been cloned and sequenced; these were shown to be the result of either partial or complete loss of P element. Of the partial reversions two mutants, whose deletion breakpoints differ only slightly from each other, retain a portion of the inverted repeat sequences from the ends of P element. Another revertant was generated by internal deletion but retains almost 400 base pairs of P element DNA. The level of activity in these revertants seems to be determined by the nature of P element sequences that remain rather than the amount of P element DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61029-02 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning and Characterization of the Vermilion Locus of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lillie L. Searles	Staff Fellow	LG, NIEHS
Others:	Robert A. Voelker	Research Geneticist	LG, NIEHS
	Mary L. Tate	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to clone the vermilion locus of Drosophila, to study the structure and expression of the gene, and to investigate the nature of vermilion mutations that can be suppressed by mutating the suppressor of sable locus.

Vermilion encodes the enzyme tryptophan oxygenase which catalyzes the first step in the synthesis of the brown eye pigment. DNA from this locus was cloned from a mutant containing a P element insertion at vermilion by "transposon tagging." The cloned DNA hybridizes to the polytene chromosome band that contains vermilion. Furthermore, DNAs from a number of vermilion mutants show detectable aberrations in the region homologous to the cloned DNA.

Several spontaneous mutations at vermilion, sable, purple, and speck are suppressible by mutations at suppressor of sable. Using cloned vermilion DNA probes, we have determined that these suppressible vermilion alleles are DNA insertion mutations, and DNAs from several of these mutants have been cloned. The mutants v^1, v^2 and v^K are insertions of the copia-like element known as 412. The weakly suppressible mutant v^{36f} contains an insertion of the element known as roo or B104. Work is continuing to determine the manner in which these insertions disrupt gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61030-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular analysis of the Om mutator in Drosophila ananassae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LG, NIEHS
Others:	Antony E. Shrimpton	Visiting Fellow	LG, NIEHS
	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hinton (1984) described an unusual mutator in D. ananassae which he speculated was a transposable element with previously unknown properties; namely the element could only be phenotypically detected by its effect on eye morphology at 15 non-pleiotropic, non-dosage compensated, non-random loci. This element was known to originate from an X chromosome in a particular stock, ca;px. With the recovery of a spontaneous singed mutation, sn^{9g}, in an ocullar morphology (Om) mutant line derivative, Om(1D)9g, it was possible to investigate his speculation. D. melanogaster singed, sn, DNA probes were used to isolate and recover a D. ananassae singed gene. A comparison of sn^{9g} and wild type singed restriction map implicated a 6½ kb insert as the element responsible for the mutator effect. This insert was shown by Southern blot and in situ hybridization to be repetitive and dispersed.

A total of 186 recombinant lines from four X-linked Om loci were examined. 80 were Om and 106 were non-Om; in all instances an in situ hybridization signal, when probed with sn^{9g} insert, was found at appropriate locations on the polytene chromosomes. Linkage was complete and showed that the sn^{9g} insert was homologous to sequences localized at the sites of Om mutants.

The most likely hypothesis is that the Om element is inserting at a target site encoded in a control sequence found at eye morphology genes. We intend to study further this phenomenon and to check this hypothesis by characterizing the Om element and insertion target sites. Of particular interest is the possibility of elucidating further the nature of eukaryotic gene structure and control, as well as to increase our knowledge of the nature of mobile genetic elements and their role in spontaneous mutation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61031-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Population Genetics of Transposable Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.H. Langley	Research Geneticist	LG, NIEHS
Others:	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS
	Clara S. Millis	Guest Worker	LG, NIEHS
	Shiu L. Huang	Guest Worker	LG, NIEHS

COOPERATING UNITS *(if any)*
Dr. Shiu L. Huang and Clara S. Millis
Environment Health Research and Testing Inc.
Research Triangle Park, N. C.

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The types of gene structural changes causing deficiency of hypoxanthine guanine phosphoribosyl transferase (HGPRT) activity in spontaneous mutations is being examined in cultured human fibroblasts. The deficiency of this enxyme activity causes a human disease (Lesch-Nyhan Syndrome). The restriction enzyme cleavage patterns of HPRT gene sequences in mutant lines will be analyzed. The work is presently focused on obtaining a large number of independent spontaneous mutants that existed in new born baby foreskins. Ten independent mutants have been isolated from different human subjects. The mutant cells were grown to large numbers. Portions of cultured cells were frozen in liquid nitrogen for cytogenetic and enzymology studies at a future time; while portions of cultured cells were frozen for DNA extraction. Southern blot analysis is now in progress to assess the possible involvement of DNA rearrangements in spontaneous mutation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61032-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure-function of mammalian lactate dehydrogenase isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The complete primary structure of human LDH-A4 isozyme has been determined by sequence analyses of LDH-A cDNA and protein. Amino acid sequence analysis of mouse LDH-A4 isozymes had positively identified 80% of the 331 residues, and the remaining amino acids were aligned on the basis of peptide compositions and homology with other known LDH sequences. Partial amino acid sequences of LDH-B4 isozymes from human and mouse have also been determined by amino acid sequencing. The amino acid sequences of 100% of the 330 residues from mouse testicular LDH-C_4 and 84% of rate LDH-C_4 have been determined. Sequence comparison among mammalian LDH isozymes clearly indicates that A_4 (muscle) and B_4 (heart) isozymes shows a closer evolutionary relationship to each other than either to the C_4 (testis) isozyme. Recently, LDH-A_4 isozymes have been found to be single-stranded DNA binding proteins which may play important roles in DNA replication, repair and recombination. The relationship of the protein structure to multiple functions as enzyme and as DNA binding protein will be further studied by *in vitro* directed mutagenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61033-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Analysis of Genetic Variation in lens crystallin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Loren C. Skow	Sen. Staff Fellow	LG, NIEHS
Others:	Maria K. Donner	Fogarty Fellow	LG, NIEHS
	Shu-Mei Huang	Research Associate	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To facilitate our studies of mouse eye mutants, we have been searching among inbred strains for genetic variation in lens crystallin genes using cDNA probes for α-, β-and γ- crystallins. Southern blot analysis of α-crystallin sequences has detected three restriction fragment size classes (Acry-1^{a,b,c}) among 42 inbred strains of mice. The polymorphisms are due to insertions flanking the structural gene. Gene mapping experiments using 69 recombinant inbred strains demonstrated close linkage of Acry-1 to H-2K on chromosome 17. Estimated recombination distance is 1.4 cM. The Acry-1 -H-2 linkage was confirmed by analysis of 18 H-2 congenic and recombinant congenic strains, in which the presence of donor Acry-1 was completely concordant with the presence of donor H-2K. The Acry-1 - H-2 association has been maintained during the development of inbred strains and suggest that strong linkage disequilibrium may exist for these genes in natural populations. In only 1 of 42 strains tested was the H-2 haplotype not predictive of Acry-1 alleles. Conservation of genes in the MHC region of mice and humans implies that the human α-crystallin gene is located on chromosome 6.

Analysis of β-crystallin sequences (Bcry) by 17 endonucleases has failed to detect restriction polymorphisms in DNA from representative strains of mice.

In contrast, γ-crystallin sequences (Gcry) have demonstrated restriction polymorphisms with all eight endonucleases so far employed. Genetic mapping experiments have been conducted on the γ-crystallin sequences using 48 recombinant inbred strains. We have found no recombination between Gcry and a γ-crystallin electrophoretic variant (LEN-1) previously mapped to chromosome 1 very close to the mutant gene, Elo, which produces anophthalmia in mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65021-12 LG

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of Germinal Mutation Induction in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. M. Johnson	Research Geneticist	LG, NIEHS
Others:	L. C. Skow	Senior Staff Fellow	LG, NIEHS
	M. L. Snell	Bio. Lab. Technician	LG, NIEHS
	Marjo Smith	Postdoctoral Fellow	LG, NIEHS
	D. P. Lovell	Statistician	BIBRA
	S. E. Lewis	Senior Geneticist	RTI

COOPERATING UNITS *(if any)*
Research Triangle Institute, Life Sciences Group, Research Triangle Park, N.C.; British Industrial Biological Research Association, Carshalton, Surrey, England

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to detect natural and induced mutations in mice for the purpose of providing understanding of the specific molecular events involved in germinal mutation and the effects of these events on the life, form and function of the mammalian organism. Results are relevant to cases of human exposures to mutagens and the potential for increased risk of genetic disease that may accompany mutagen exposure. The problem is approached by detecting mutations at specific biochemical loci with electrophoretic methods, by conducting characterization studies on the mutant genes and gene products, and by examining the animals for expressed physical abnormalities correlated with mutation rate increases and with specific induced-mutant genotypes. The methods have led to successful identification of more than 20 ethylnitrosourea-induced mutants affecting proteins such as malic enzyme, α hemoglobin and phosphoglucomutase, but there is little evidence to suggest that most of the mutants are detrimental to health-related characteristics. The results raise questions as to the extent hypotheses of human genetic risk based upon increased mutation rates are indicative of elevated probabilities for significant genetic damage. As some mutagens are therapeutically beneficial the possibility of false risk estimates and unjustified restrictions on mutagen exposures raise important medical and legal considerations. Previously published mammalian-genetic data are being reexamined to determine if new interpretations are possible, and laboratory experiments involving the use of other mutagens and more powerful detection techniques are planned for the future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65029-03 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Mouse Lens Mutations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Loren C. Skow Sen. Staff Fellow LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 61033-01 LG entitled "Molecular Analysis of Genetic Variation in lens crystallin".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65032-02 LG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Induced Mutations at the MOD-1 and GPI Loci of Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. V. Malling	Research Geneticist	LG, NIEHS
	J. G. Burkhart	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project Number Z01 ES 65033-01 LG entitled "In Vivo Mammalian Mutagenesis".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65033-01 LG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vivo Mammalian Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. V. Malling	Research Geneticist	LG, NIEHS
	J. G. Burkhart	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*

C.A. Hutchinson III, M.H. Edgell, & S.C. Hardies
UNC at Chapel Hill
Chapel Hill, N.C.

E. J. Eisen
North Carolina State University
Raleigh, N.C.

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.3	2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is to study gene mutations and small intrachromosomal changes directly in exposed mammals. We have taken two approaches. 1). Site specific alterations in proteins detected in single cells: Antigenic differences between rat and mouse LDH-C were used to detect mutations in mouse sperm. Initial work indicated that this system had potential for the study of mutagenesis in mammals; later research showed that the apparent mutation frequency varied too much to give reproducibly useful data. The conclusion was reached that although antibodies had the needed sensitivity, interspecies differences may not be appropriate as markers for induced mutations; also, a model mutant cell must exist for a proper test of antibodies. Therefore, use of antibodies are being applied to spontaneous and induced mutants of malic enzyme (MOD-1) in mice. Normal (MOD-1) was purified and antibodies produced; sperm and somatic cells were labeled with this antibody. A mutant form of MOD-1 was purified. The future strategy is to produce and test antibody to the abnormal MOD-1 using cells from normal homozygotes and heterzygotes with the mutant allele. 2). Site specific changes detected at the DNA level: A major problem for detection of genetic damage directly in mammalian DNA is that most genes occur in one, or few copies; there are however, two possibilities. The first is the use of mitochondrial (mt) DNA. Cloned mouse mtDNA has been used for restriction analysis of sperm mtDNA isolated from a single mouse; after additional technical improvements, the mtDNA from treated mice will be examined for mutations. The second is the use of viral DNA transformed into mammalian DNA. Double stranded DNA from ϕX174 am3, cs70 was transformed into mouse L-cells. The DNA was incorporated into several places in tandem arrangements. Using restriction enzymes and ligase it was possible to transfect spheroplasts with ϕXDNA from the transformed mammalian cells. Attempts are being made to create a mouse strain with ϕXDNA in the genome for the study of mutation induction in any part of the animal tissue.

LABORATORY OF MOLECULAR BIOPHYSICS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10003-05 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthetic and Analytical Studies in Bioorganic Chemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Oscar Hernandez	Senior Staff Fellow	LMB	NIEHS
OTHER:	M.B. Gopinathan	Visiting Fellow	LMB	NIEHS
	Michael Walker	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Behavioral and Neurological Toxicology
Development and Reproductive Toxicology

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio Organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to develop chemical methods and apply these to specific biological areas. Two problems of current interest are 1) chemistry of glutathione and its adducts with epoxides; 2) mechanisms of separation and purification of polypeptides by high-performance liquid chromatography. The level of success of a program on bio-mechanisms, such the one posed by the glutathione pathway, is largely dependent on the ability to generate the chemical information necessary for the interpretation of biological experiments. A systematic program was initiated and eventually has culminated in the development of unequivocal syntheses of the requisite compounds, methods of separation for diastereoisomeric conjugates of glutathione with alkene and arene oxides, establishment of the absolute configuration of these thioether stereoisomers, and correlation of synthetic compounds with enzymatically produced conjugates. The high-performance liquid chromatography analysis of peptides has focused on the role of hydrophobicity in the separation mechanism by reversal phase liquid chromatography. Experiments have been designed and implemented which alter the intrinsic hydrophobicity of peptide molecules during chromatographic analysis. These changes are introduced in a predictable fashion such that valuable structural information is derived from conventional reversed-phase experiments. Successful applications of this approach include: characterization of amphibian peptides in small-cell carcinoma, purification of a protein seemingly synthesized in response to estrogen exposure, and characterization of two forms of epidermal growth factor isolated from mouse submaxillary gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES10004-05LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Nuclear Magnetic Resonance (NMR) Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Anthony Ribeiro	Chemist	LMB	NIEHS
	Louis Levy	Research Chemist	LMB	NIEHS
	Halasya Ramanathan	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the biophysical nuclear magnetic resonance (NMR) program is the elucidation of the mechanisms by which chemicals and heavy metals present in the environment produce toxic effects in living systems. The use of NMR methodology in achieving this objective may be considered to fall into two broad categories: 1) In vivo metabolic analysis using NMR spectroscopy. Such studies probe the metabolism of chemicals and heavy metals in various tissues and organs directly when sufficient concentrations are present to permit detection. Additionally, studies of the effects of these agents on intermediary metabolism, the metabolism of high energy phosphate compounds, and on the pyridine nucleotide reduction charges are carried out when the levels of chemicals/heavy metals are well below the direct detection threshold, but can still lead to a toxic response as reflected in perturbations of those paramters. 2) In vitro studies of molecular structure and dynamics using NMR spectroscopy. Such studies are aimed both at obtaining a more complete understanding of the solution structure of chemical toxins, and toward the analysis of the mode of interaction of various toxic substances with presumed or demonstrated biological targets (e.g., nucleic acids, enzymes, etc.). As a consequence of the need to use specific labeling (including carbon-13 and fluorine-19 labeling) in connection with many of these NMR studies, the program includes a synthetic component, the goals of which are closely interfaced with the needs of the program. Finally, it is noted that an overriding motivation for this program is the evaluation and demonstration of the extent to which non-invasive analysis by NMR can be used to follow the progress of a toxic response in real time and with individual experimental animals, thereby leading to more efficient use of these animals in toxicity studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER:

Z01 ES 10007-04 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High Pressure Liquid Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carol E. Parker	Research Chemist	LMB	NIEHS
OTHER:	J. Ronald Hass	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Research Service Branch

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 50080-02 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 10012-02 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Design of Laboratory Data Management System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Mike P. Moorman Biomedical Engineer LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Mass Spectrometry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25012-05 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Lung as an Endocrine Organ Controlling Intravascular Thrombosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Roberta McMillan	Biologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 80008-10 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-13 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analytical Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB,	NIEHS
Other:	J. Ronald Hass	Research Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	Kun Chae	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

EPA; Dow Chemical Company; Monsanto; New York Department of Health; University of Umea, Sweden; Health Prot. Br., Canada; National Fisheries Laboratory; Wright State University; University of Nebraska.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	1.7	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major effort this year has been concerned with development, validation, and application of methods for the accurate and reliable determination of chlorinated dibenzo-p-dioxins and dibenzofurans at low levels in environmental samples. Emphasis has been placed on accurate quantification and highly specific qualitative identification. Methods for extraction and cleanup of soil, adipose tissue, and liver have been developed and refined. A collaborative, study has been started involving ten laboratories in three countries, to compare the analytical capabilities of these laboratories (which all use different techniques and approaches) for the low part-per-trillion level measurement of a series of dioxins and furans in human adipose tissue. The most reliable analytical approaches should be identifiable as a result of this study. Analysis of animal tissues and soil for 2,3,7,8-TCDD was used in another study to evaluate the bioavailability of TCDD bound to soil from Times Beach, Missouri.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30015-10 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Gaseous Ion Chemistry.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Ronald Hass	Research Chemist	LMB
OTHER:	Maurice M. Bursey	Visiting Scientist	LMB
	Earl White	Chemist	LMB
	Mike Kinter	Chemist	LMB
	Donald J. Harvan	Chemist	LMB
	Carol E. Parker	Chemist	LMB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The unimolecular and collision-induced decompositions of $(NaF)2Na^+$ ions formed by excitation of a sodium fluoride surface with 7 keV Xe ion was investigated. By comparing the results with ab initio calculations, it was possible to demonstrate the presence of two structures of this cluster ion, differing in their internal energy content. The lower energy state (i.e., more stable) was similar to that of the crystal structure. By application of a tandem quadruple mass spectrometer system, the conversion of kinetic energy into internal energy was investigated for the molecular ion of 2-pentanone. It was found that between 14 and 25% of the available kinetic energy was converted into internal energy. Onset of conversion to internal energy through an electronically excited state was observed at 2.0 eV. In a second study, it was demonstrated that, at least for $[C_4R_4]^+$ ions, there exists a Hammett correlation for ratios of intensities of collision-induced fragments on the basis of the statistical theory of mass spectra and in practice. To help define the gas-phase reaction paths leading to the formation and subsequent decomposition of PO_3^-, the negative chemical ionization mass spectra of a group of dimethylvinyl and dimethylaryl phosphates were obtained, employing a variety of mass-spectral techniques. In every case, a major pathway to PO_3^- appears to consist of the sequence (a) capture of a thermal electron by the molecule, (b) loss of the vinyl or aryl group to produce the dimethyl phosphate anion, and (c) elimination of dimethyl either to yield PO_3^-. This ion, when suitably activated, decomposes further only by losing an oxygen atom to yield PO_2^-, the metaphosphite anion. The mechanism for the substitution reactions of NH_3 in the ammonia chemical ionization mass spectra of aromatic esters has been investigated. The formation of the NH_3 adduct ion involved the protonated molecule and the formation of an unstable intermediate which then underwent a reactive collision with another NH_3 to give the adduct product ion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30020-13 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transport and Metabolism of Phthalate Esters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
OTHER:	Richard Philpot	Research Chemist	LP	NIEHS
	J. Ronald Hass	Research Chemist	LMB	NIEHS
	Robert London	Research Physicist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Gary Liss, M.D., CDC, NIOSH
P.K. Seth, Ph.D., Lucknow, India
B. Lake, Ph.D., B.I.B.R.A., UK

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.1	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The undesirable biological effects of phthalate esters (the most ubiquitous of all environmental pollutants), which include acute testicular atrophy resulting in male sterility, hepatocarcinogenesis in rats and mice, and proliferation of peroxisomes, are mediated through their metabolites. We have identified some 27 metabolites of the most widespread phthalate, di(2-ethylhexy)phthalate (DEHP), have postulated a metabolic pathway for their formation, have confirmed and detailed the first two steps in the pathway, and have formed an hypothesis as to the part of the pathway responsible for the dramatic differences in metabolic profiles in different species. As the biological activities of the different metabolites are elucidated, it becomes clear that species differences in metabolism can potentially result in resistance of non-rodent species to the undesirable biological effects. This makes extrapolation of toxicity tests from rodents to man highly unreliable, even though we have also provided evidence for general internalized exposure of humans to phthalate esters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30034-08 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry of Aromatic Compounds and Their Environmental Transformation Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Levy	Research Chemist	LMB	NIEHS
OTHER:	A.R.K. Murthy	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 10004-05 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30050-08 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical and Enzymatic Conjugation of Glutathione with Epoxides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Oscar Hernandez	Senior Staff Fellow	LMB	NIEHS
OTHER:	M.B. Gopinathan	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Marine Pharmacology Section, LP, NIEHS and TRTP.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the most important pathways available to organisms for the detoxication of potentially harmful chemicals is via reaction with glutathione. Among the numerous transformations involving alkylating reactions of glutathione, its reaction with epoxides was selected for thorough examination. This choice was dictated by our interest in the mechanism of biosynthesis of epoxides and the implication of these compounds as direct acting toxic agents. The project consisted of two phases, a chemical and an enzymatic component, for which the following objectives were defined: 1) develop methodology for the study of glutathione reactions with epoxides; 2) establish the stereochemical fate of these transformations; 3) elucidate the mechanism(s) of the enzyme catalyzed reaction of glutathione with epoxides. The first objective was accomplished with the development of methods which allow the preparation of diastereomerically pure glutathione conjugates as well as sensitive methods for the analysis of such compounds. The stereochemical course of the epoxide ring opening step was established with the aid of optically pure epoxides. This knowledge was translated into stereochemical profiles whereby diastereomer identification on a reversed-phase liquid chromatography system became possible. Experiments with various epoxide substrates and hepatic glutathione transferases from the little skate and the rat showed remarkable stereochemical consistency. In both cases, a majority of the purified enzymes showed a high preference for attack-addition of thiol at the R-carbon on the epoxide ring. Other enzymes showed the opposite preference, i.e. addition to the S-carbon. A mechanism was postulated to explain the stereoselectivity of this enzymatic transformation and based on this mechanism a model was proposed for the active site geometry of three glutathione transferases. The use of stereochemical profiles as a way of assesing differences, or similarities, among glutathione transferases is currently being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30051-08 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Specific Binding Toxic Polyhalogenated Aromatic Hydrocarbons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James D. McKinney	Supervisory Research Chemist	LMB	NIEHS
OTHER:	Philip W. Albro	Research Chemist	LMB	NIEHS
	Kun Chae	Chemist	LMB	NIEHS
	E.E. McConnell	Veterinary Pathologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 30066-08 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30064-07 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analytical Methodology for Environmental Health Sciences.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Ronald Hass	Research Chemist	LMB
OTHER:	Donald J. Harvan	Chemist	LMB
	Reiner G. Stoll	Expert	LMB
	Charles N. McEwen	Expert	LMB
	Carol E. Parker	Chemist	LMB
	Ann Richard	Chemist	LMB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new ion source, using negative primary ions, for liquid secondary ion mass spectrometry (LSIMS) was designed and constructed. The most significant advantages were that 10 kV power supplies (compared to 20 kV for positive sources) were adequate, resulting in simpler design requirements and reduced electrical arcing in the source. A highly focused point source was fitted to a double focusing mass spectrometer which was also equipped with a moveable target. An ion optical system was designed and constructed so that secondary ions formed on a flat surface could be extracted and injected into the mass spectrometer. Intense secondary ion currents (~10^{-11} to 10^{-12} amps) were obtained from organic compounds, despite the power dissipation of ~10^{+4} watts per cm^2. By use of a piezoelectric-effect crystal motor system and microcomputer, it was possible to move the sample reproducibly in 10^{-8} m steps (10 nm) in two directions. Surface distributions of organic chemicals could be obtained with a spatial resolution <1 micron. The idea of chloride attachment negative chemical ionization (NCI) mass spectrometry was extended to HPLC/MS. The world's first tandem high resolution mass spectrometer was built to our specifications and installed. This instrument permits simultaneous high mass resolution on both parent and daughter ions. Of perhaps greater environmental health interest, moderate resolutions can be achieved on both parents and daughters at good sensitivity. Initial studies have demonstrated the power of this instrument for the characterization of peptides, corticosterones and phospholipids from biological sources. Advantages include greatly increased analytical information and a much reduced susceptibility to sample contamintion. In addition to the new tandem instrument, the use of kinetic energy release (KER) measurements has been extended from its traditional role in the study of gaseous ion chemistry to the characterization of unknown organic compounds. A new time-of-flight design has been developed which offers resolution equal to or better than that achieved when magnetic instruments are operated at very high mass (i.e.>8000 daltons).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30065-07 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Mass Spectral Reactions in Field-Free Regions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Ronald Hass	Research Chemist	LMB	NIEHS
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 30064-07 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30066-08 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Theoretical Basis and Molecular Mechanisms of Biological Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. McKinney	Sup. Research Chemist	LMB	NIEHS
Others:	T. Darden	Staff Fellow	BRAP	NIEHS
	L. Birnbaum	Research Microbiologist	NTP	NIEHS
	J. Lamb	Research Biologist	NTP	NIEHS
	K. Chae	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

L. Pederson	UNC, Chapel Hill, N.C.
S. Oatley	LMB, Oxford, England
C. Blake	LMB, Oxford, Englad
R.E. Coleman	Duke University, Durham, N.C.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Halogenated aromatic hydrocarbons constitute a broad class of compounds with varying structure and toxicity. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the prototypical structure, is the most toxic compound of this type. Theoretical, quantitative structure activity relationship (QSAR) and experimental studies focusing on polychlorinated biphenyls (PCBs) as models have established the importance of molecular polarizability and receptor to PCB separation distance in binding to the dioxin or Ah receptor. The work suggests a new stacking type model for the Ah receptor which has universal applicability to the range of binding-structures observed. Further theoretical and experimental studies suggested that Ah receptor binding is a necessary but not sufficient condition for toxicity and a second receptor must be proposed to account for the structural requirements of planarity and halogenation in toxicity, as opposed to induction. A protein binding model consistent with the structural requirements for toxicity has been identified. Molecular modeling work, competitive binding assays and several in vivo studies have demonstrated the importance of thyroxine binding proteins in toxicity and suggested that the high toxicity of TCDD is the expression of potent and persistent thyroid hormone activity. A molecular mechanism is proposed involving a two protein-receptor model in which the planar aromatic system controls the initial receptor binding and halogen substitutents control subsequent nuclear events. This mechanistic hypothesis is being further investigated for its relevance in dioxin toxicity as well as insight into the mechanism of thyroid hormone action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-06 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Chemically Induced Photosensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Colin F. Chignell	Chief, LMB	LMB	NIEHS
OTHER:	Ann G. Motten	Staff Fellow	LMB	NIEHS
	Krysztof Reszka	Visiting Fellow	LMB	NIEHS
	Robert D. Hall	Staff Fellow	LMB	NIEHS
	Garry Buettner	NIH Senior Postdoctoral Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Enrico Gratton, Dept. of Physics, University of Illinois, Champaign, Urbana, IL.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.1	4.1	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Light is known to interact with endogenous or exogenous chemical agents in the skin or eyes, to produce photosensitization (phototoxicity or photoallergy). While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanisms of sensitization are unknown. The objective of this study is to determine whether light-induced free radicals or active oxygen species play a role in photosensitization. Benoxaprofen [2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid] is an anti-inflammatory drug that causes acute phototoxicity in many patients. Irradiation of benoxaprofen in anerobic organic solvents provided evidence for hydrogen abstraction reactions and a drug-derived carbon centered radical. In the presence of oxygen both superoxide and singlet oxygen were detected. Oxygen markedly increased the photohemolysis of human red blood cells by benoxaprofen suggesting that oxygen-derived species were involved. Inhibition of photohemolysis by the anti-oxidant butylated hydroxyanisole emphasized the importance of free radicals in this process. Anthracene, a component of coal tar, is a potent photosensitizing agent both in vivo and in vitro. Irradiation of anthracene in aerated ethanol produced singlet oxygen. Anthracene caused a concentration dependent photohemolysis of human erythrocytes that was markedly enhanced by the presence of oxygen and was abolished by β-carotene, a singlet oxygen quencher. These findings are consistent with the generation of singlet oxygen by irradiated anthracene, which in turn reacts with membrane lipids to produce peroxides that are responsible for membrane damage and, ultimately, hemolysis. Irradiation of the following chemical agents in aqueous or organic solvents also produced free radicals and/or active oxygen species: benzoxazole, 2-methylbenzoxazole, 2-phenylbenzoxazole, musk ambrette, chlorpromazine, furosemide and fluoranthene. These photoinduced species may play an important role in the phototoxic and photoallergic properties of these agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50077-02 LMB

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Free Radical Intermediates of Antiparasitic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert Docampo	Guest Worker	LMB	NIEHS
OTHER:	Silvia N.J. Moreno	Visiting Fellow	LMB	NIEHS
	Ronald P. Mason	Research Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Molecular Biophysics

SECTION: Molecular Biophysics

INSTITUTE AND LOCATION: NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Parasitic diseases are the most widespread of all the major human diseases. Effective treatment is nonexistent for many of them, and most of the available drugs have been shown to have mutagenic, cytotoxic, and carcinogenic activities. Recently it has become increasingly apparent that many reactive intermediates of xenobiotics are free radicals. The formation of free radicals, including oxygen-derived radicals, may lead to extensive cellular damage. The consequences of radical-initiated reactions may be the immediate death of the cells or may be more subtle and delayed as evidenced by the development of neoplasms. In a study of Trypanosoma cruzi (the agent of Chagas' disease), crystal violet, a triarylmethane dye widely used by blood banks to eliminate transmission of Chagas' disease by transfusion, was found to undergo a one-electron reduction to produce a carbon-centered free radical. Both radical formation and the trypanocidal action of crystal violet, were enhanced by light. The photodynamic action of rose bengal on T. cruzi has also been established. These observations may have important chemoprophylactic implications, since illumination of blood to be transfused may result in a better therapeutic ratio. The free radical intermediates ($O_2^{\cdot-}$, $OH^{\cdot}$) generated by human polymorphonuclear leukocytes in the presence of antibody-coated T. cruzi were identified by ESR spectroscopy. These radicals may play an important role in host resistance and/or pathogenesis of Chagas' disease. The ability of Tritrichomonas foetus (the agent of trichomoniasis in cattle) hydrogenosomal and cytosolic fractions to generate metronidazole and nitrofuran anion radicals was established. These results support the role of air oxidation as a detoxification reaction for the metronidazole anion radical and the involvement of a ferredoxin in its formation. On the other hand, redox cycling of nitrofurans, with formation of high steady state concentrations of oxygen-derived radicals, might be of toxicological significance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50078-02 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radical Anion Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Silvia N.J. Moreno	Visiting Fellow	LMB	NIEHS
	Roberto Docampo	Guest Worker	LMB	NIEHS

COOPERATING UNITS *(if any)*

Clinical Pharmacology, VA Hospital, Minneapolis, MN

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to determine the role played by free radicals in the metabolism of xenobiotics. The anaerobic incubation of almost all nitroaromatic xenobiotics, e.g., nitrobenzene, with the microsomal, mitochondrial, or cytosolic fractions of rat liver in the presence of either NADH or NADPH, leads to a multiple-line electron spin resonance spectrum characteristic of the nitro anion free radical. We have now demonstrated nitro anion radical formation by mitochondria using endogenous cofactors. Nitro drugs do not affect mitochondrial respiration, in particular the coupling to ADP. The sites of nitro reduction, as determined by inhibitors of the mitochondrial transport chain, appear to be NADH dehydrogenase and outer-membrane NAD(P)H cytochrome c reductase.

Halogen-substituted nitro compounds are radiosensitizers and are among the most toxic nitro compounds. Loss of halide by the nitroaromatic anion forms a very reactive carbon-centered free radical, as detected by spin trapping. which reactswith cellular macromolecules. The irreversible binding of these nitro compounds to DNA, protein, etc. was inhibited by spin traps.

Free radical formation by hepatic microsomal cytochrome P-450 reduction of gentian violet, SO_2 and O_2 has also been investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50079-02 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Free Radical Metabolite Formation by Peroxidases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Volker Fischer	Visiting Fellow	LMB	NIEHS
	Paul West	Guest Worker	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this investigation are to detect quantitate and identify free radical metabolites generated from endogenous and exogenous chemical by peroxidase enzymes. Electron spin resonance (ESR) investigations of the prostaglandin hydroperoxidase and a model enzyme system, horseradish peroxidase, have demonstrated the enzymatic formation of free radical metabolites. The oxidation of benzidine and several derivatives was studied using horseradish peroxidase and prostaglandin synthase. Benzidine was metabolized to a radical cation and a charge-transfer complex composed of the benzidine and its two electron (di-imine) oxidation product. The two-electron oxidation product, the di-imine, is a resonance structure of the nitrenium ion, the proposed ultimate carcinogenic metabolite of aromatic amines. Mono-acetylbenzidine is a relatively poor peroxidase substrate. Electron spin resonance spectroscopy, employing a millisecond time scale fast-flow method, has revealed the formation of a transient phenoxy radical in the reaction of acetaminophen with horseradish peroxidase/H_2O_2 and bovine lactoperoxidase/H_2O_2. The short-lived radical is clearly distinguished from the persistent paramagnetic melanin polymers that are generated by prolonged incubation of acetaminophen in the presence of oxidizing enzymes. Ram seminal vesicles and acetaminophen under fast-flow conditions demonstrated the oxidation of acetaminophen to its phenoxyl free radical by the mammalian enzyme prostaglandin hydroperoxidase. Sulfur-centered free radicals have been detected when cysteine was incubated with horseradish peroxidase and H_2O_2. In the presence of either molecular oxygen or hydrogen peroxide the thiyl radical was converted to the cysteine sulfonic and sulfinic acids. Reduced glutathione (GSH) was also oxidized to a sulfur-centered radical (GS·) by horseradish peroxidase and H_2O_2. Since cysteine and glutathione play an important role in the structure and function of sulfhydryl-containing proteins, these oxidation reactions may modulate the biological function of these compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-02 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Environmental Health Applications of Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Ronald Hass	Research Chemist	LMB
OTHER:	G. Dean Marbury	Chemist	LMB
	Carol E. Parker	Chemist	LMB
	Donald J. Harvan	Chemist	LMB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

More than 2000 sample analyses were performed in support of other NIEHS scientists. Collaborative research problems have included: characterization of metabolites of phthalates and diethylstilbestrol, measurement and identification of chlorinated dibenzo-p-dioxins and dibenzofurans in soil and animal tissues; estimation of 3-methoxy-4-hydroxyphenylglycol in rat brain; identification of physalemine-like peptides and epidermal growth factor in tissues; identification of corticosterone metabolites in mammary gland; and characterization of phospholipids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50081-02 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Improvements in Fluorometric Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert D. Hall	Staff Fellow	LMB	NIEHS
OTHER:	Colin F. Chignell	Chief, LMB	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Z01 ES 50046-06 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50082-01 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Tumor Promoters and Antipromoters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
Other:	Ronald P. Mason	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tumor promoters can often be differentiated by their effects in two model systems: polynuclear aromatic hydrocarbon (PAH)-initiated carcinogenesis in mouse skin and nitrosamine-initiated carcinogenesis in regenerating rat or mouse liver. Phorbol esters promote in both systems, some phthalate esters promote the latter and fail to affect the former, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) promotes the latter and is an antipromoter for the former, while polychlorinated biphenyls (PCBs) may promote or act as an antipromoter for both depending on the dosages tested. A comparison of these classes of environmental pollutants may provide mechanistic insights absent when any one is studied by itself. This is a new project that is still largely in the planning and preparation stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-10 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Roberta McMillan	Biologist	LMB	NIEHS
	David Henke	Guest Worker	LMB	NIEHS
	Serge Kouzan	Visiting Fellow	LMB	NIEHS
	Marc Reilly	Chemist	LMB	NIEHS
	Roger Nolan	Visiting Fellow	LMB	NIEHS
	Jorg Schrieber	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

R. Boucher	Associate Professor	Dept. Medicine	UNC
Arnold Brody	Staff Fellow	LPFT	NIEHS

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.7	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulmonary tissue produces high amounts of prostaglandins (PG), leukotrienes (LT), and hydroxy fatty acids (HFA) in response to a number of stimuli or pathological states. These lipids have diverse biological activities. The goal of this study is two-fold: first to develop an understanding of factors that control the biosynthesis of these lipids and second to determine their role in the secretory and inflammatory processes of the lung.

We have chosen to study the biosynthesis of these lipids in dog tracheal epithelial cells and to determine their role in control of Cl^- and mucus secretion. Dog trachea cells make primarily PGD_2 and a number of LTs. LTC_4, LTB_4 and two unknown LTs are also produced. While Cl^- secretion appears to be primarily under control of PGD_2 formation, other data suggest a regulatory role for the LTs. We intend to characterize further the LTs formed and relate them to Cl^- secretion.

We are also examining the formation of LTs in response to asbestos exposure. Cultures of rat pulmonary macrophages release LTs in response to asbestos exposure. Macrophages release primarily LTB_4 but some LTC_4 is also liberated. We intend to characterize further the products released by asbestos from rat macrophages and to explore the possible relationship between macrophages and the development of asbestosis.

We have also found that mouse skin is very rich in prostaglandin synthase and essentially devoid of lipoxygenase activity. The major cyclo-oxygenase product is PGE_2 as characterized by high pressure liquid chromatography and gas chromatography-mass spectrometry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-08 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas Eling	Research Chemist	LMB	NIEHS
OTHER:	Jeff Boyd	Biol. Lab. Technician	LMB	NIEHS
	Ronald Mason	Research Chemist	LMB	NIEHS
	Gregory Reed	Staff Fellow	LMB	NIEHS
	Robert Krauss	Biologist	LMB	NIEHS
	John Curtis	Chemist	LMB	NIEHS
	Marc Reilly	Chemist	LMB	NIEHS
	Jorg Schrieber	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Drs. Fouts and Anderson, Laboratory of Pharmacology; and Dr. L. Marnett, Wayne State University

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long range goal of this project is to study the oxidation of chemicals to toxic metabolites by prostaglandin synthetase (PHS) and to demonstrate the significance of this system in chemically-induced toxicity or carcinogenesis. We have shown that PHS converts both polycyclic hydrocarbons and aromatic amines to mutagens as measured by bacterial tester systems. Other in vitro studies have demonstrated the formation of electrophilic metabolites that react with macromolecules. Benzo(a)pyrene-7,8-diol is metabolized to an anti-diol epoxide by PHS. We have compared PHS and NADPH-dependent metabolism in hamster trachea and human bronchial explants. In both tissues, stimulation of PHS increased anti-diol epoxide formation. We have also shown that the anti-inflammatory drug phenylbutazone is converted by PHS to a phenylbutazone peroxyl radical which can epoxidize BP-7,8-diol. This represents a new mechanism for the metabolism of chemicals by PHS. The aromatic amine carcinogen 2-aminofluorene (2-AF) is metabolized to free radical intermediates by PHS. The stable end products are azo-, nitro-fluorene and 2-aminodifluorenylamine. We have studied the formation of phenolic 2-AF adducts and obtained evidence that 2-AF is oxidized to several free radicals or free radical derived products (nitrenium ion). These radicals may not only be responsible for covalent binding to DNA but also may indeed be the proximate carcinogenic and mutagenic agents. We have also studied the formation of 2-AF DNA adducts catalyzed by PHS. Several unique 2-AF-DNA adducts were detected. Our studies indicate that PSH activates chemicals to ultimate carcinogenic metabolites which may be of importance in the initiation of tumors in extrahepatic tissue. Thus PHS is an enzyme system that, like cytochrome P-450, is important in the metabolism of xenobiotics.

LABORATORY OF PHARMACOLOGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 35005-05 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

*Carcinogen-Induced DNA Damage and Repair (New title)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marshall W. Anderson	Mathematician	LP	NIEHS
Other:	Dr. Felix Romagna	Visiting Fellow	LP	NIEHS
	Dr. Steve Belinsky	NRS Fellow	LP	NIEHS
	Dr. Steven Reynolds	Staff Fellow	LP	NIEHS
	Ms. Jill Stowers	"P" Appointment	LP	NIEHS

COOPERATING UNITS *(if any)*

Drs. John Bend and Richard Philpot, Laboratory of Pharmacology; Dr. Steve Strom, Duke University; Dr. Robert Maronpot, National Toxicology Program; and Dr. Stewart Aaronson, National Cancer Institute

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is compelling evidence that many mutagens and carcinogens are able to react with cellular DNA either directly or following metabolic formation of reactive products. If DNA replication proceeds on such a modified template before altered bases or nucleotides are removed by enzymic repair processes, the mutations can be genetically fixed. Thus, the extent of carcinogen-induced promutagenic DNA damage and the capacity of cells to repair such damage represent critical events in the initiation of carcinogenesis. We are studying the in vivo formation and repair of carcinogen metabolite-DNA adducts in tissues and cells that are susceptible or resistant to carcinogen-induced neoplasia. We are concerned with the effects of dose of carcinogen on the amounts and types of adducts formed and on the subsequent repair of these adducts. Our studies on the in vivo formation and repair of benzo(a)pyrene (BP) metabolite-DNA adducts in a variety of tissues and cell types emphasize the possibility that long-term exposure to low levels of BP could result in the accumulation of BP-DNA adducts in cells which have slow turnover rates. Even if environmental exposure to BP (and other polycyclic aromatic hydrocarbons) is too small to induce neoplasia, the persistence of BP-DNA adducts may produce aberrations in transcripts of genetic information in various cell types and lead to other toxic effects. We have developed several techniques to study in vivo repair of carcinogen-induced DNA damage. 1) An in vivo - in vitro to measure unscheduled DNA synthesis and 2) a nucleoid assay to detect carcinogen-induced changes in DNA superhelicity as well as study of time-dependent repair of such damages.

(*Formerly titled "Pharmacokinetic Considerations in the Formation and Repair of Carcinogen-DNA Adducts)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70132-05 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intestinal Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Schiller	Research Chemist	LP	NIEHS
Other:	C.R. Shoaf	NIH-Postdoctoral Fellow	NRSA	NIEHS
	M.W. King	NIH-Postdoctoral Fellow	NRSA	NIEHS
	D.E. Chapman	Toxicologist	UNC	NIEHS

COOPERATING UNITS *(if any)*
Curriculum of Toxicology, University of North Carolina, Chapel Hill, N.C.

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our research focuses on the development and use of animal model systems to study the regulation of gastrointestinal functions. Of particular concern are the regulation of intestinal absorption and metabolism of nutrients, and the alteration of these normally occurring events in response to oral exposure to biologically active environmental toxins. Currently, our investigations involve the systematic examination of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) on normal lipid assimilation. The mechanism of the physiologic changes is monitored with a combination of in vitro and in vivo techniques. In particular, our studies include examination of 1) chylomicra formation, transport and metabolism, 2) mesenteric lymph chylomicra and very low density lipoproteins, 3) dose-related responses to TCDD exposure and 4) genetic component of metabolic response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70200-10 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms for Regulating the Intracellular Bioavailability of Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)*

PI:	B.A. Fowler	Research Biologist	LP	NIEHS
Other:	P. Mistry	Visiting Fellow	LP	NIEHS
	P. Goering	NRSA Postdoctoral Fellow	LP	NIEHS

COOPERATING UNITS *(if any)*

I. Armitage, Department of Molecular Biophysics, Yale University, D.H. Petering, Department of Chemistry, University of Wisconsin-Milwaukee, C.F. Chignell, Laboratory of Environmental Biophysics

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Molecular mechanisms which regulate the intracellular bioavailability of metals such as lead and cadmium have been studied in both mammals and marine invertebrates. High affinity cytosolic lead-binding proteins PbBP of 63,000 (63K) and 11,500 (11.5K) daltons from kidneys of rats have been partially purified by gel and anion exchange chromatography, electrophoresis, and sucrose density gradient analysis. These molecules were found to exhibit dissociation constants (K_d) for lead of 10^{-8}M. Sucrose density gradient analysis (SDGA) of these molecules showed sedimentation coefficients of 2S and 4.6S for the 11.5K and 63K dalton PbBP, respectively. Competitive binding studies on sucrose density gradiantes with cytosol showed displacement of ^{203}Pb by Pb^{II}, Cd^{II} and Zn^{II} ions but not Ca^{II} ions. Cell-free nuclear translocation studies showed both time- and temperature-dependent uptake of ^{203}Pb from kidney cytosol and KCl extraction of these nuclei followed by SDGA indicated the presence of one saturable peak with a sedimentation coefficient of 2S. Cd^{II} and Zn^{II} ions effectively blocked the nuclear uptake of ^{203}Pb. The data indicate that these high affinity PbBP, which act as the initial cytosolic ligands for Pb in the kidney, are capable of mediating the intranuclear translocation of Pb and that this process is competitively blocked by Cd^{II} and Zn^{II}. The 11.5K dalton protein, but not the 63K protein, was also found to regulate the inhibitory effects of Pb on the heme biosynthetic pathway enzyme δ-aminolevulinic acid dehydratase (ALAD). Reversal of Pb-induced inhibition of hepatic ALAD activity was dependent on the concentration of 11.5K dalton PbBP added to the reaction mixture. Kinetic analysis of either hepatic or renal ALAD activity indicated a non-competitive inhibition pattern. Addition of the semi-purified 11.5K dalton PbBP to the assay mixture markedly reduced the inhibitory effects of Pb on the Vmax of the enzyme from either tissue. The data indicate that the 11.5K dalton protein confers partial resistance to Pb inhibition of liver ALAD in vitro and suggests a similar role for this protein in kidney with respect to the resistance of renal ALAD to Pb inhibition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80001-12 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microsomal Mixed-Function Oxidase Systems: Specificity and Function

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	R.M. Philpot	Research Chemist	LP	NIEHS
Other:	B.A. Domin	Staff Fellow	LP	NIEHS
	R.R. Vanderslice	Graduate Student	LP	NIEHS
	Z. Parandoosh	Visiting Fellow	LP	NIEHS
	G. Carver	Biologist	LP	NIEHS

COOPERATING UNITS *(if any)*

Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, CA

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For a number of years we have been investigating the cytochrome P-450 monooxygenase systems (P-450 systems) of rabbit liver and lung. The results of these studies have provided explanations for some of the differences that have been noted between the abilities of these two tissues to metabolize xenobiotics. The key to these explanations has been the discovery that the concentrations and types of cytochrome P-450 isozymes present in liver and lung differ significantly. At present we are primarily concerned with the distribution of cytochrome P-450, form 5, in liver and lung and its response to various inducers and repressors. Until recently, the evidence for isozyme 5 in the liver could be obtained only by immunochemical methods. Now, however, this enzyme has been purified from the livers of rabbits treated with phenobarbital. A direct comparison of isozyme 5 from liver and lung can now be carried out. This structural, immunochemical and catalytic comparison should provide information sufficient to establish if these proteins are identical. The second major isozyme of rabbit lung, form 2, is also under investigation. The results of our previous work have not provided any evidence that the liver and lung enzymes are different. However, we now have some evidence that microheterogenous forms of isozyme 2 may exist in the liver but not in the lung. The immunochemical techniques now available make detailed investigations of the P-450 systems of tissues other than liver and lung (the two tissues with the highest concentrations of cytochrome P-450) possible. Because one of the isozymes (form 5) studied in our laboratory is highly active in the metabolism of aromatic amines, we have initiated an investigation of the P-450 system of rabbit bladder, the target tissue for the carcinogenic effects of these compounds. Rabbit bladder containes isozymes 2, 5 and 6 but does not contain isozyme 4. Therefore, the bladder has the enzymes required for the metabolism of aromatic amines to reactive products. Like the lung, however, the bladder has little or no ability to N-hydroxylate 2-acetylaminofluorene and can activate this compound only after is has been deacetylated to 2-aminofluorene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80002-13 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymes Metabolizing Chemicals: Effectors of These Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James R. Fouts Research Pharmacologist LP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Cell Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project combined with Z01 ES 80003-10 LP and continued as Z01 ES 80039-01 LP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80003-10 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Xenobiotic-Metabolizing Enzyme Activity in Skin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dr. James R. Fouts Research PharmacoloP NIEHS

COOPERATING UNITS *(if any)*

Biometry Branch, Histology

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Cell Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project combined with Z01 ES 80002-13 LP and continued as Z01 ES 80039-01 LP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80007-13 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjugation and Oxidation Pathways for Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John R. Bend	Chief	LP	NIEHS
Other:	E. Cheung/J. Horton	Visiting Fellows	LP	NIEHS
	L. Dostal	NIH-Postdoctoral Fellow	LP	NIEHS
	R. Brigelius	Visiting Associate	LP	NIEHS
	D. Brier	Chemist	LP	NIEHS
	C. Harris	Biologist	LP	NIEHS
	C. Serabjit-Singh	Chemist	LP	NIEHS
	O. Hernandez	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Arrhenius Laboratory of Biochemistry, Stockholm University; Laboratory of Molecular Biophysics, NIEHS; Laboratory of Pulmonary Function and Toxicology, NIEHS; Department of Biochemistry, Vanderbilt University

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.4	2.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epoxides are frequently formed as metabolites of unsaturated hydrocarbons by cytochrome P-450-dependent monooxygenase activity. Many arene and alkene oxides are known to react covalently with macromolecules and to transform cells in vitro, suggesting they are ultimate carcinogens, mutagens or cytotoxins. We are studying various aspects of the enzymatic formation and subsequent metabolism of epoxides in relationship to cell-selective and organ-selective toxicity of compounds metabolized to epoxides by both hepatic and extrahepatic tissues. Particular attention is given to the respiratory tract because this is a common site for pollutant-mediated damage. We are currently investigating stereochemical aspects of the P-450-dependent oxidation of styrene to styrene 7,8-oxide in microsomal and reconstituted monooxygenase systems, stereochemical and kinetic aspects of the biotransformation of model alkene (styrene 7,8-oxide) and polycyclic arene (benzo(a)pyrene 4,5-oxide) oxide substrates by cytosolic and purified glutathione transferases of diverse origin, the distribution and characteristics of components of the cytochrome P-450-containing monooxygenase system in vascular endothelium and the status of the tripeptide glutathione, which is important in detoxication of electrophilic metabolics, in perfused lung, Clara cells, alveolar type II cells, alveolar macrophages and tracheal cells (mixed) isolated from rabbit lung before and after exposure to electrophilic metabolites or conditions of oxidant stress (e.g., exposure to paraquat).

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-08 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Altered Membrane Function in Xenobiotic Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.B. Pritchard	Research Physiologist	LP	NIEHS
Others: S.-H. Lee	Expert Research Biochemist	LP	NIEHS
G.-J. Neufeld	Expert Research Biochemist	LP	NIEHS

COOPERATING UNITS *(if any)*

University of Florida, C.V. Whitney Laboratory

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ability to transport solutes across epithelial membranes is vital for the function of many organs, e.g. secretion and reabsorption by the kidney. In turn, epithelial transport depends upon the coordinated function of individual transport systems located at opposite poles of the cells in the apical (BBM) and basolateral (BLM) membranes. Many of these membrane processes, particularly for anions, are not yet understood. Furthermore, because of their complex organization, functional importance, and exposed location, epithelial membranes are particularly susceptible to toxic effects of foreign chemicals. Our major recent emphasis has been on increasing our understanding of vectorial solute transport in polar epithelia, including the properties of specific carrier systems, the driving forces energizing transport and the coupling of events at opposite poles of the cells. Results for both organic (glucose, amino acids, organic acids) and inorganic (Na^+, Cl^-, $SO_4^=$) solutes emphasize the complexity of the chains of events which lead to active solute transport by epithelial -- thus indicate that there are multiple sites for potential disruption by xenobiotics. Moreover, they also show that similar chains are responsible for transport of widely different solutes; thus, that the same mechanism may be responsible for breakdown in transport of unrelated solutes. For example, the organ cation, L-lysine, and the inorganic anion $SO_4^=$, were shown to be transported via pH gradient-dependent mechanisms. Therefore, transport of both solutes could be markedly inhibited by the protonophore, pentachlorophenol, which collapses the pH gradient across the membrane.

GPO 964 917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80032-07 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

*Excretion and Toxicity of Xenobiotics to Marine and Terrestrial Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: J.B. Pritchard Research Physiologist LP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

*This project combined with project Z01 ES 80031-08 LP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80038-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Suicide Inhibitors of Cytochrome P-450: Isozyme and Tissue/Cell Selectivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John R. Bend	Chief	LP	NIEHS
Others:	J. Mathews	Staff Fellow	LP	NIEHS
	L. Dostal	NIH-Postdoctoral Fellow	LP	NIEHS
	G. Parker	Chemist	LP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microsomal monooxygenase systems contain multiple isozymes of cytochrome P-450 which contribute differentially to the oxidative metabolism of endogenous and exogenous substrates; isozyme differences in K_m, V_{max}, regioselectivity and stereoselectivity are common. Hence, modulation of the relative amounts of various P-450 isozymes can have pronounced effects on chemical metabolism and toxicity. For this reason we are studying isozyme selectivity and tissue/cell selectivity of suicide inhibitors of cytochrome P-450. The suicide inhibition by 1-aminobenzotriazole (ABT) and some of its novel N-alkylated derivatives, which we synthesized and characterized, is being studied in rabbit lung and liver. Although ABT is a potent suicide inhibitor, it shows little P-450 isozyme selectivity. N-benzyl-ABT, on the other hand, is both potent and highly selective (but still not specific; it destroys isozymes 2 and 6 associated enzyme activity but does not significantly affect isozyme 5 catalyzed activity). Synthesis of N,N-disubstituted-ABT derivatives is in progress and these compounds, once available, may demonstrate enhanced isozyme selectivity. In a related project, the chemical nature of the alkylbenzene metabolites, which selectively destroy pulmonary (versus hepatic) cytochrome P-450, and the biochemical nature (e.g., isozyme specificity, involvement of other enzymes and cofactors) of the pathways involved are being elucidated. Results to date demonstrate at least two distinct pathways for metabolic activation, one relying only on the presence of NADPH for suicide destruction, the second relying both on the presence of NADPH and alcohol dehydrogenase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80039-01 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Xenobiotic Transformation in Isolated Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James R. Fouts	Research Pharmacologist	LP	NIEHS
Others:	Theodora Devereux	Research Biologist	LP	NIEHS
	Thomas Massey	Visiting Fellow	LP	NIEHS
	Janet Diliberto	Biological Lab. Technician	LP	NIEHS
	Thomas Eling	Research Chemist	LMB	NIEHS
	Richard Philpot	Research Chemist	LP	NIEHS

COOPERATING UNITS *(if any)*
Biometry and Risk Assessment Program (BRAP); Histology, NIEHS

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.2	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major cell types of the lung and skin are being isolated ans studied for their metabolisms of xenobiotics and selected lipids (in comparison with liver cells). Special studies are being made with Clara, type II and ciliated cells of the lung and sebaceous and basal cells of the skin. Studies of the effects of the techniques used in isolating the enriched populations of cells from tissues are being made with various antibodies to selected cytochrome P-450 isozymes and Western blotting/microdots for quantifying these isozymes and related peptides in cells at various stages in the isolation procedures. A microspectrophotofluorometer is being used to quantify xenobiotic metabolism in single cells and to study variations in this metabolism among cells of an homogenous population. Variations in enzyme activity in periportal and centrolobular liver cells in the perinatal period are compared with these activities in the adult liver. Isolated skin cells are being used to study the metabolism of prostaglandins and arachidonic acid and the site of accumulation of lipid soluble chemicals like the PCBs.

(Combined with former projects -- Z01 ES 80002-13 LP and 80003-10 LP)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80040-01 LP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Pharmacogenetics of Liver Microsomal Cytochrome P-450s

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	M. Negishi	Visiting Scientist	LP	NIEHS
Others:	Y. Kato	Visiting Associate	LP	NIEHS
	N. Harada	Visiting Fellow	LP	NIEHS
	B. Burkhart	Biologist	LP	NIEHS
	T. Zoucha	Stay-in-School	LP	NIEHS

COOPERATING UNITS *(if any)*

City of Hope, California

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Liver microsomal steroid hydroxylation activities, which are catalyzed by specific forms of cytochrome P-450s, are known to exhibit sex-dependent expression. In inbred mice, the expression of steroid hydroxylations differs from one to another strain. It has been known that 16α-hydroxylation activity of female mice is recessively regulated by a single gene between 129/J and C56BL/6J. This genetic regulation, however, between 129/J and BACB/CJ was found to be autosomal dominant, indicating that more than one allele are involved in this regulation. The sex-dependent expression of hydroxylases in adulthood are irreversibly predetermined by neonatal androgens. It is reasonable to assume that these differences of microsomal cytochrome P-450s in sex may effect sex-dependent drug toxicity, including chemical carcinogenesis. To understand the biological phenomena surrounding sex-dependent and developmental regulation of gene expression in steroid hydroxylases, we have previously purified female predominant mouse testosterone 15a-hydroxylase ($P\text{-}450_{15\alpha}$). Now male predominant mouse testosterone 16α-hydrocylase has been purified and characterized. A specific antibody against $P\text{-}450_{15\alpha}$ or $P\text{-}450_{16\alpha}$ was raised in rabbits, and they completely inhibited only 16α- or 15α-hydroxylation activity. Liver polysomes bearing $P\text{-}450_{15\alpha}$ or $P\text{-}450_{16\alpha}$ mRNA were precipitated by the antibodies from which the cDNA banks were constructed. By the selective hybridization to ^{32}P-labeled probe synthesized from the immunoenriched mRNAs, the cDNAs encoding $P\text{-}450_{16\alpha}$ has been isolated. By the further characterization and isolation of genomic clones with these cDNA probes, we hope to understand the mechanisms of sex- and age-dependent gene expression of these hydroxylases, and gain insight into the role of cytochrome P-450s in steroid metabolisms and sex differences of chemical toxicity. We have also isolated and characterized cDNAs for drug-induced mouse liver microsomal cytochrome P-450. A comparison between androgen-dependent and xenobiotic-dependent cytochrome P-450 genes will provide useful and better information to understand mechanisms of gene expression.

GPO 914-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80041-01 LP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Detection and Quantitation of Cytochrome P-450 Isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.M. Philpot	Research Chemist	LP NIEHS
Others:	B.A. Domin	Staff Fellow	LP NIEHS
	P. Bent	Biological Lab. Technician	LP NIEHS

COOPERATING UNITS *(if any)*
Department of Biochemistry and Drug Metabolism, Hoffmann-LaRoche, Nutley, N.J.

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Elucidation of the factors responsible for the substrate specificities of various cytochrome P-450 monooxygenase systems (P-450 systems) requires the means to detect and quantitate the different isozymes of cytochrome P-450. With this information and knowledge of the catalytic activities of the P-450 isozymes, the metabolic capacity of a given P-450 system can, to a great extent, be explained. Techniques for the detection and quantitation of P-450 isozymes have been developed and are being applied to a number of problems. However, these techniques have brought into question the substrate specificities that have been established for some of the enzymes. The reason for this is the sensitivity of the immunochemical methods. In the past, the criterium for the purity of an isozyme preparation used to determine substrate specificity was the appearance of the preparation following electrophoresis and staining for protein. If no impurities were observed, the preparation was judged to be "homogenious". With immunochemical methods we have show that up to 50% contamination with a second P-450 isozyme cannot be detected by protein staining. Such contamination can, however, contribute significantly to the substrate specificity of the preparation. With immunochemical methods we have described a number of major differences between the monooxygenase systems of rabbit liver and lung. The major isozymes of rabbit lung (forms 2 and 5) are minor forms in the liver unless the animals have been treated with phenobarbital. Treatment of rabbits with polycyclic aromatic hydrocarbons results in increases of two isozymes in the liver but only one in the lung. These differences and others are now being investigated in a number of different species including humans. With antibodies to both rabbit and rat isozymes of cytochrome P-450, we have been able to demonstrate that all species examined exhibit similar differences between liver and lung and that all species have analogous isozymes of cytochrome P-450.

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25001-07 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Mutagenesis in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. C. Barrett	Research Chemist	LPFT, NIEHS
Others:	T. Hesterberg	Postdoctoral Fellow	LPFT, NIEHS
	M. Oshimura	Expert	LPFT, NIEHS
	N. Tanaka	Visiting Fellow	LPFT, NIEHS
	P. Lamb	Biological Lab. Tech.	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Environmental Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most chemical carcinogens induce DNA damage and are mutagenic at specific genetic loci; however, certain carcinogens (including the human carcinogens diethylstilbestrol, asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the activity of these chemicals, proposed as carcinogenic but not mutagenic, to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo cells in culture. Diethylstilbestrol (DES) induced cell transformation in the absence of mutations at specific genetic loci but was an inducer of aneuploidy by nondisjunction. DES was found to disrupt spindle microtubule organization in the cells and to cause chromosome loss and gain. Cell transformation and aneuploidy induction by DES were similar in terms of dose response and cell-cycle dependence. Furthermore, colcemid, a well known inducer of nondisjunction was also found to induce neoplastic transformation of cells in culture, confirming that aneuploidy induction is a potential mechanism for cell transformation. The mechanism of action of two other human carcinogens, asbestos and arsenic, were studied. Asbestos and other mineral fibers, including fiberglass, induced cell transformation in a dose dependent manner. The ability of fibers to induce cell transformation depended on fiber dimension, i.e., long, thin fibers were most potent. These findings indicate that this experimental system is an useful model to study the mechanism of fiber-induced tumorigenesis since similar results are found for fiber size dependence for mesothelioma induction in animals. An excellent correlation was observed between the induction of cell transformation and chromosome changes, including numerical and structural aberrations, suggesting that asbestos causes cell transformation by affecting chromosome stabilty. Arsenic also induced cell transformation and caused chromosome aberrations. The results with different carcinogens indicate that a significant effect of carcinogens is on chromosome number and structure and that these changes are important in the genesis of carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25002-07 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Carcinogenesis and Promotion Studies with Respiratory Tract Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. Nettesheim Chief LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project No. Z01 ES 25023-01 entitled "Studies on the Mechanism of Neoplastic Development in Airway Epithelial Cells."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25007-06 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Deposition of Inhaled Particles and Pathogenesis of Initial Pulmonary Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: A. R. Brody Research Biologist LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Study continued under Project No. Z01 ES 25024-01 entitled "Pathogenesis of Early Pulmonary Lesions Induced by Inhaled Inorganic Particles."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25008-06 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macrophage Accumulation and the Initial Chemotactic Stimulus Activated by Asbestos

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. R. Brody Research Biologist LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Pulmonary Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project No. Z01 ES 25025-01 entitled "Asbestos Activation of Complement-Dependent Chemotactic Factors for Macrophages."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25009-05 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Membrane Interactions with Chrysotile and Crocidolite Asbestos

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. R. Brody	Research Biologist	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project No. Z01 ES 25026-01 entitled "Interactions of Inorganic Particles with Pulmonary Cell Membranes."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25014-02 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Cell-Cell and Cell-Substratum Interactions in Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. M. Jetten Senior Staff Fellow LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Cell Biology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project No. Z01 ES 25021-01 entitled "Differentiation and Differentiative Functions of Tracheal Epithelial Cells."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25017-02 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Proliferation and Its Relation to Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. M. Jetten Senior Staff Fellow LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Cell Biology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study continued under Project No. Z01 ES 25022-01 entitled "Study of the Molecular Mechanisms of Action of Retinoids."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25020-02 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. E. R. Hook	Research Chemist	LPFT, NIEHS
Others:	L. A. Dethloff	Biological Lab. Tech.	LPFT, NIEHS
	L. B. Gilmore	Biologist	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulmonary surfactant is a complex mixture of lipids and proteins that prevents collapse of the alveoli and distal airways at low lung volumes. In the lungs surfactant exists within two major compartments referred to, respectively, as the intracellular and extracellular pools. The most potent inducer of the pulmonary surfactant system reported appears to be silica dust. The objectives of this work are to elucidate mechanisms through which silica stimulates surfactant production in the lungs. Extracellular surfactant from the lungs of rats was obtained by bronchoalveolar lavage. Intracellular surfactant was isolated from lavaged lungs by density gradient centrifugation of the homogenized tissue. Intra- and extracellular pools of surfactant phospholipids were 0.69±0.27 mg/pair of lungs and 1.21±0.09 mg/pair of lungs, respectively. Intratracheal injection of silica produced marked changes in both the intracellular and extracellular pools of surfactant, an effect that was both time- and dose-related. Twenty-eight days following a single 50 mg dose of silica, the intra- and extracellular pools of surfactant were increased 80-fold and 29-fold, respectively. Expansion of the surfactant pools could be achieved through alterations in the dynamic equilibrium that under normal circumstances exists between the intracellular and extracellular compartments. This hypothesis was examined in vivo using ^{3}H-palmitate as a phospholipid precursor. Biosynthesis of surfactant phospholipids was increased approximately 10-fold but transfer to the extracellular pool increased only 4-fold thus accounting for the expansion of the intracellular pool of surfactant. Although secretion increased 4-fold the turnover of phospholipids in the extracellular pool increased only 2-fold thereby leading to expansion of the extracellular pool. These studies demonstrate that silica causes a large increase in the biosynthesis of surfactant phospholipids and that the expansion of the intracellular and extracellular pools is due to imbalances in the dynamic equilibrium between the two compartments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25021-01 LPFT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Differentiation and Differentiative Functions of Tracheal Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. M. Jetten	Sen. Staff Fellow	LPFT, NIEHS
Others:	J. Rearick	Staff Fellow	LPFT, NIEHS
	H. Smits	Visiting Fellow	LPFT, NIEHS
	M. E. Porter	Biol. Lab. Tech.	LPFT, NIEHS
	M. Deas	Chemist	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Cell Biology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For the study of the regulation of mucin synthesis and secretion hamster tracheal epithelial cell cultures grown on collagen gels appear to provide us with a good model system. Cells were radiolabelled with ^{3}H-glucosamine and/or ^{35}S-sulfate and their secretory products were characterized. Mucins were identified on the basis of their high molecular weight and complete resistance to proteoglycan-degrading enzymes, ruling out proteoglycans. Labelling with several monosaccharide precursors and analysis of the high molecular weight products by strong acid hydrolysis or neuraminidase treatment showed the presence of sialic acid but not mannose. β-Elimination released oligosaccharides with the conversion of most of the N-acetylgalactosamine into N-acetylgalactosaminitol, indicating that the oligosaccharides are O-glycosidically linked with protein. This mucin has been further characterized via β-endogalactosidase treatment and it was shown that R-GlcNacβ1-3Galβ1-R linkages are present. This finding is supported by lectin affinity chromatography on DSA-agarose gels. Studies on the action of retinoids and monensin on mucin secretion are in progress. For the study of the regulation of differentiation rabbit tracheal epithelial cell cultures are used as a model system. These cells undergo terminal differentiation into squamous, cornifying cells when reaching confluency. This process is inhibited by retinoids and promoted by calcium and serum. Several biochemical changes accompany this change in phenotype. Undifferentiated cells produce large amounts of hyaluronic acid whereas in keratinizing cells the synthesis of hyaluronic acid is very much reduced. Cells undergo quantitative as well as qualitative changes in keratin proteins as shown by 2-D electrophoresis and by immunoblot analysis using monoclonal antibodies. A 48 kd keratin appears to be a good marker for this differentiation. Differentiation into mucin secretory cells can be induced when cells are grown on collagen gels in the presence of retinoic acid. Retinoic acid appears to have a key role in the control of differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25022-01

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Molecular Mechanisms of Action of Retinoids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. M. Jetten	Senior Staff Fellow	LPFT, NIEHS
Others:	J. E. Shirley	Biological Lab. Tech.	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Cell Biology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retinoids affect many biological and biochemical properties of eukaryotic cells. Their mechanism of action is unclear. One possibility is that they alter gene expression either indirectly or directly via the mediation of the binding protein and interaction with the chromatin. The induction of ornithine decarboxylase (ODC) activity by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) appears to be a good tool to study the mechanism of action of retinoids. Retinoids can affect the induction of this enzyme at several levels. We have no evidence that ODC activity is affected by retinoids at a post-translational level. Transglutaminase appears not to be involved and no evidence of an inhibitory substance activated or induced by retinoic acid was detected. Retinoids could prevent ODC induction by blocking the interaction of TPA with its receptor. We have ruled out this possibility since retinoic acid does not compete with the binding of ^{3}H-PDBu. We have demonstrated that this receptor, which appears to be the protein kinase C, is causal in inducing ODC, since diacylglycerols are able to induce its activity. Retinoic acid does not interfere with the protein kinase C activity. These resultsindicate that retinoic acid acts at a step after the activation of protein kinase C. This could be by interfering with the process of transmitting the signal from protein kinase C to the nucleus or by blocking transcription directly via binding to specific sites on the chromatin. The structure-function relationships of the ability of retinoids to inhibit ODC induction and the capacity to bind to the binding protein suggest a role for these proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25023-01 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Mechanism of Neoplastic Development in Airway Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Nettesheim	Chief	LPFT, NIEHS
Others:	J. C. Barrett	Research Chemist	LPFT, NIEHS
	D. J. Fitzgerald	Visiting Fellow	LPFT, NIEHS
	M. J. Mass	Former Postdoctoral Fellow	LPFT, NIEHS
	D. G. Thomassen	Former Postdoctoral Fellow	LPFT, NIEHS
	T. E. Gray	Biologist	LPFT, NIEHS
	M. P. Smith	Biological Lab. Tech.	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.1	2.1	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on mechanisms of neoplastic development in airway epithelial cells are being conducted with the aim to characterize pre-neoplastic epithelial cell variants on both the cellular and molecular level. By investigating important modulators of neoplastic progression, we hope to elucidate its cellular and biochemical basis.

An analysis of cell populations of transformed rat tracheal epithelial (RTE) cells indicates that transformed stem cells produce large numbers of cells not exhibiting transformed growth characteristics.

Studies on the role of the anchorage independent (ag^+) phenotype in neoplastic transformation of RTE cells indicates that ag^+ cell variants are being generated in preneoplastic RTE cell populations at a rate of about 10^{-4} per cell, per cell generation and that the ag^+ phenotype may not be an obligatory step in neoplastic transformation of RTE cells.

Chromosomal analyses of RTE cell transformants suggest that spontaneous and induced transformants have similar qualitative and quantitative chromosomal changes which might be important in the establishment of the EG-variant phenotype.

The tumor promoter TPA does not increase the frequency of the EG-variant phenotype but it may enhance the development of the ag^+ phenotype in some but not other preneoplastic RTE cell clones.

Retinoic acid inhibits RTE cell transformation at concentrations which are not inhibitory for growth of normal RTE cells. Whether RA inhibits the clonal expansion or the expression of RTE cell transformation is under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25024-01 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Early Pulmonary Lesions Induced by Inhaled Inorganic Particles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. R. Brody	Research Biologist	LPFT, NIEHS
Others:	L. H. Hill	Chemist	LPFT, NIEHS
	V. Roggli	Guest Worker	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interstitial fibrotic lung disease is commonly found in humans exposed occupationally and environmentally to certain inorganic particles. We have established animal models to elucidate the initial cellular events associated with inhalation of the toxic dusts, asbestos and silica, and we have determined that a variety of inorganic particles, regardless of shape or concentration, deposits initially at bifurcations of alveolar ducts. It is at these sites of particle deposition that we have documented the earliest anatomic alterations induced by inhalation of asbestos fibers. These alterations at duct bifurcations include (1) phagocytosis of asbestos fibers by the alveolar epithelium, (2) translocation of fibers through the epithelium to capillary spaces and interstitial connective tissue, (3) phagocytosis of fibers by interstitial fibroblasts, (4) formation of interstitial intracellular microcalcifications, and (5) accumulation of alveolar and interstitial macrophages. Now, we report the following: (1) One month after a 1-hr exposure to chrysotile asbestos, the alveolar duct bifurcations exhibited normal epithelial cells, but there were significant increases in the numbers of interstitial fibroblasts and macrophages and in the amount of interstitial extracellular matrix, some of which was collagen. (2) Autoradiographic studies demonstrated that increased numbers of epithelial cells lining terminal bronchioles as well as epithelial and interstitial cells of alveolar duct bifurcations incorporated tritiated thymidine into DNA. (3) One month after the 1-hr exposure to asbestos, 19% of the fibers which had originally been deposited after inhalation was still present in the interstitium of the rats' lungs. Further studies are ongoing to establish the role of interstitial fibers and the mechanisms through which the fibers induce fibroblast proliferation and collagen production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25025-01 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Asbestos Activation of Complement-Dependent Chemotactic Factors for Macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold R. Brody	Research Biologist	LPFT, NIEHS
Others:	D. B. Warheit	Postdoctoral Fellow	LPFT, NIEHS
	L. H. Hill	Chemist	LPFT, NIEHS
	G. George	Visiting Fellow	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In earlier studies, we showed that pulmonary macrophages migrate to the sites where inhaled chrysotile asbestos fibers initially are deposited (i.e., surfaces of alveolar duct bifurcations). These macrophages form a major component of an early asbestos-induced interstitial lesion in rats. Thus, in order to establish the basic cellular mechanisms of asbestos-induced lung disease, it is essential to determine the chemical mediators which attract macrophages to these sites of fiber deposition. Chrysotile asbestos fibers used in vitro activate complement proteins in peripheral blood serum and in lavaged cell-free lung proteins. After brief inhalation of chrysotile asbestos, fluids lavaged from the lungs of exposed rats contain substantial chemotactic activity for macrophages compared to fluids from sham-exposed animals ($p < .01$). We hypothesize that this chemotactic activity is derived from complement activated by inhaled asbestos on alveolar surfaces. This contention is supported by the following observations: (1) Production in vitro of chemotactic activity by asbestos in serum or in lung lavageates was blocked by complement inhibitors. (2) Fractionation, by molecular sieve chromatography, of serum proteins and concentrated proteins lavaged from the lungs of asbestos-exposed rats showed that chemotactic activity was detected in the 14-18,000 MW range. This fractionation profile is similar to C5a, the chemotactic product of complement activation. In addition, (3) rats treated with cobra venom factor to deplete circulating complement as well as complement-deficient mice demonstrated significantly depressed macrophage accumulation at sites of asbestos deposition. Pulmonary macrophages are the cells which form the initial inflammatory response to asbestos inhalation. Our findings support the hypothesis that macrophages are attracted to the anatomic sites where inhaled asbestos fibers activate complement-derived chemotactic activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25026-01 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interactions of Inorganic Particles with Pulmonary Cell Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold R. Brody	Research Biologist	LPFT, NIEHS
Others:	L. H. Hill	Chemist	LPFT, NIEHS
	J. E. Gallagher	Graduate Student	LPFT, NIEHS
	G. George	Visiting Fellow	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inhaled particles such as asbestos and silica are toxic to pulmonary cells. In recent studies on mechanisms of membrane injury, we have shown that chrysotile asbestos causes damage to erythrocyte membranes through binding to terminal sialic acid (SA) residues. The hemolytic events involved (1) binding of the positively-charged chrysotile fibers to negatively-charged SA groups, (2) rapid (within 5 min) distortion of the cells, (3) redistribution of SA groups, and (4) alterations of intracellular Na^+, K^+ ratios. Negatively-charged crocidolite asbestos bound to and distorted red cells but had no effect on SA groups or ion flux. To establish whether or not similar mechanisms of membrane injury play a role in particle-induced toxicity of pulmonary cells, we have extended our studies to pulmonary macrophages. Our hypothesis is that non-specific (i.e., non-receptor mediated) binding and subsequent uptake of positively-charged particles are mediated by negatively-charged cell surface sialic acid groups. In support of this hypothesis we have shown the following: (1) Wheat germ agglutinin (WGA), a lectin which binds to sialic acid, is distributed evenly across macrophage surfaces. (2) Positively-charged carbonyl iron (Fe) spheres and chrysotile asbestos fibers bind to macrophage membranes at 4° C and the binding is blocked by a dose-dependent pretreatment of the cells with WGA. Other lectins such as Ricin and ConA do not inhibit binding at comparable doses. (3) Removal of cell-surface glycoproteins with neuraminidase and periodate inhibit binding of the positively-charged particles. (4) Fe-spheres normally are readily phagocytized by macrophages at 37° C. In the presence of WGA, over 90% of the phagocytic activity is blocked, but other lectins have no effect. These studies support our hypothesis that charged surface sialic acid groups play a role in particle binding and phagocytosis. Further studies are ongoing to substantiate our findings using negatively-charged particles and membrane markers of receptor turnover.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25027-01 LPFT

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Identification and Characterization of Materials Secreted by Pulmonary Clara Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. E. R. Hook	Research Chemist	LPFT, NIEHS
Others:	S. E. Patton	Postdoctoral Fellow	LPFT, NIEHS
	A. M. Jetten	Senior Staff Fellow	LPFT, NIEHS
	P. Nettesheim	Chief	LPFT, NIEHS
	L. B. Gilmore	Biologist	LPFT, NIEHS
	L. A. Dethloff	Biological Lab. Tech.	LPFT, NIEHS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Pulmonary Function and Toxicology

SECTION: Biochemical Pathology Group

INSTITUTE AND LOCATION: NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The functions of the nonciliated bronchiolar epithelial cell (Clara cell) of the lungs are not known. Numerous morphological investigations in many species have led to the hypothesis that the Clara cell is secretory although the nature of those secretions and their function in the airways are not known. The objectives of this research are to eludicate the secretory nature of the Clara cell, identify and characterize those secretions and determine their extracellular functions. We have developed a model system for the study of Clara cell functions and metabolism using cells isolated from the lungs of rabbits. Cells were dispersed from lung tissue using a pancreatic protease introduced via the trachea. These dispersed cells contained approximately 8% Clara cells. The Clara cells were enriched to about 40% by centrifugation on a continuous density Percoll gradient. The Clara cells were then subjected to centrifugal elutriation, resulting in further purification (Clara cells 75-85% pure). These cells were added to collagen I coated culture dishes and incubated overnight in Ham's F12 medium. The attached cells consisted of at least 90% Clara cells. The isolated Clara cells contained abundant endoplasmic reticula and osmiophilic cytoplasmic granules typical of Clara cells *in vivo*. These features were maintained when the cells were incubated for 24 hours. Incubation of the isolated cells with ^{35}S-methionine for 4 hours resulted in the radiolabelling of many intracellular proteins some of which were released into the incubation medium. The major radiolabelled protein present in the medium had a molecular weight of about 10,000 daltons as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis under reducing conditions. Clara cells could be maintained in *in vitro* culture for periods up to 2 weeks in Ham's F12 medium supplemented with insulin, transferrin, EGF, hydrocortisone, bovine hypothalamus extract and antibiotics. These studies indicate that the isolated Clara cells may be an appropriate model for the study of Clara cell function and metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25028-01 LPFT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis for Cellular Changes in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. C. Barrett	Research Chemist	LPFT, NIEHS
Others:	T. Gilmer	Senior Staff Fellow	LPFT, NIEHS
	M. Koi	Visiting Fellow	LPFT, NIEHS
	D. Thomassen	Postdoctoral Fellow	LPFT, NIEHS
	L. Annab	Biological Lab. Tech.	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neoplastic development of Syrian hamster embryo (SHE) cells is a multistep process. We have examined the influence of chemical carcinogens and the role of cellular and viral oncogenes in this process. We have compared the susceptibilities of normal and carcinogen-induced preneoplastic SHE cells to neoplastic transformation following transfection by the calcium phosphate precipitate techniques with plasmids of genomic clones of four oncogenic viruses: polyoma virus, Harvey murine sarcoma virus (Ha-MSV), Rous sarcoma virus (RSV) and MC29 virus (pSVv-myc). Normal SHE cells transfected with polyoma virus DNA formed progressively growing tumors of hamster origin within 3-4 weeks when injected into nude mice. In contrast, SHE cells treated with Ha-MSV DNA remained nontumorigenic. SHE cells treated with RSV DNA formed one tumor (one of six sites) with a latency period of 15 weeks. SHE cells transfected with either Ha-MSV DNA and pSVv-myc DNA or RSV DNA and pSVv-myc DNA formed tumors with short latency periods. Polyoma virus DNA, Ha-MSV DNA or RSV DNA could individually neoplastically transform preneoplastic SHE cells which were immortalized following treatment with the carcinogen diethylstilbestrol. These results suggest that multiple changes or activated oncogenes are required for the neoplastic transformation of SHE cells. To determine if normal cellular factors or genes can regulate the phenotypic expression of tumorigenicity and/or oncogenes, cell-cell hybrids between chemically transformed SHE cells and either normal or preneoplastic SHE cells were prepared. Our results indicate that anchorage independence, which is a good marker for tumorigenicity of these cells, is suppressed in hybrids between tumorigenic and normal cells and in hybrids between tumorigenic and most but not all preneoplastic cells. This suggests that this suppressive ability may be lost during neoplastic progression and represents one step in this process.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70010-08 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Normal and Abnormal Embryonic Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Pratt	Head, Experimental Teratogenesis Section	LRDT NIEHS
Others:	G. K. Andrews	Senior Staff Fellow	LRDT NIEHS
	R. P. DiAugustine	Research Chemist	LRDT NIEHS
	R. I. Grove	Staff Fellow	LRDT NIEHS
	C. S. Kim	IPA	LRDT NIEHS
	K. S. Morgan	NIH Postdoctoral Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*
Department of Pediatrics
University of Washington, Seattle

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
6.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research project is to understand at the morphological, cellular and biochemical levels various aspects of normal and abnormal embryonic development, especially relating to craniofacial development. Glucocorticoids are teratogenic in vivo, and our results using whole rodent embryo culture demonstrate they exert a direct effect on the embryo; day 8 embryos cultured for 48 hrs develop heart and neural tube malformations whereas day 10 embryos develop cleft lip in culture after a 48 hr exposure to triamcinolone acetonide. Cultured day 10 mouse embryos become responsive to epidermal growth factor (EGF) after 48 hrs in culture, during which time the initial stages of secondary palate formation are occurring; future studies will ascertain the effect of other growth factors and hormones. Our results indicate that maternal EGF does not cross the mouse placenta although an embryonic form of EGF appears in the conceptus on day 12 of gestation; future work will determine the site(s) of synthesis of this EGF within the embryo. A cell culture system has been developed in order to examine growth and differentiation of isolated embryonic palatal epithelial cells and EGF is an absolute requirement; the effects of EGF are enhanced in the presence of cyclic AMP. The dioxin TCDD appears to induce cleft palate by altering the differentiation of the medial palatal epithelial cells. Glucocorticoids exert their cleft palate inducing effects by inhibiting palatal mesenchymal cell proliferation; immunocytochemical studies have localized glucocorticoids receptors mainly to the palatal mesenchyme. In palatal mesenchyme cell culture, one of the major effects produced by glucocorticoids associated with growth inhibition is an alteration in the turnover of plasma membrane phosphatidylinositol; future studies will examine the effect in greater detail. An established line of human embryonic palatal mesenchymal cells, along with a liver-derived metabolic activating system, has been used to develop a short-term growth-inhibition screening test for potential teratogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70015-01 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression During Murine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. K. Andrews Senior Staff Fellow LRDT NIEHS

Others: R. M. Pratt Head, Experimental Teratogenesis Section LRDT NIEHS

COOPERATING UNITS *(if any)*
La Jolla Cancer Research Foundation, La Jolla, California

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was designed to study molecular events which may regulate gene expression during development. The work to date has focused primarily on expression of the murine alphafetoprotein (AFP) and metallothionein (MT) genes with particular emphasis on studying effects of glucocorticoids on mRNA levels in developing mice. Both AFP and MT genes are actively expressed in visceral yolk sac endoderm as well as in fetal and neonatal liver. We have found that in neonatal liver, glucocorticoids can induce a 3-5 fold decrease in AFP mRNA with a concomitant 5-10 fold increase in MT mRNA. However, during fetal development, glucocorticoids, even at teratogenic levels, did not result in alterations in AFP or MT mRNA levels in visceral yolk sac. Furthermore, hepatic AFP mRNA levels were unaffected by glucocorticoid and only after day 16 of gestation was there a small glucocorticoid-induced increase in hepatic MT mRNA. Since the actions of steroid hormones are thought to be mediated by specific receptor molecules in the target tissue, we analyzed developing visceral yolk sac and liver for glucocorticoid receptors. There was an 8-fold increase in receptor levels in visceral yolk sac (i.e. to 10,000 sites/cell) between days 9 and 14 of gestation. Receptor levels in fetal liver were constant (2-4 x 10^3 sites/cell) until day 16 and increased slightly to parturition. Hepatic receptor levels increased rapidly after birth reaching adult levels (i.e. 30,000 sites/ cell) by days 10-14 post-partum. These results indicate that receptor levels may be limiting the glucocorticoid responses in early stages of fetal liver development, whereas, in visceral yolk sac, presence of receptor alone is not sufficient for response, at least as measured by changes in AFP and MT mRNA levels. Heavy metals (i.e. Cd^{+2}, Zn^{+2}, Cu^{+2}) were found to induce an increase in MT mRNA in neonatal liver without affecting AFP gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70060-11 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Biology/Toxicology of Estrogenic Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS
Others:	R. R. Newbold	Biologist	LRDT NIEHS
	K. S. Korach	Research Endocrinologist	LRDT NIEHS
	Y. Tomooka	Visting Fellow	LRDT NIEHS
	J. C. Barrett	Research Biologist	LPFT NIEHS
	R. P. DiAugustine	Research Chemist	LRDT NIEHS
	C. T. Teng	Expert	LRDT NIEHS

COOPERATING UNITS *(if any)*
Bowman-Gray School of Medicine
Duke University Medical Center
Medical Foundation of Buffalo
University of Würzburg
University of Texas Medical Center at Houston

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.6	2.8	2.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have continued to determine the molecular forms and cellular targets of estrogenic chemicals and establish the mechanisms by which interactions of estrogens with developing genital tract target cells result in permanently altered differentiation, including neoplasia. In the period covered by the report, the ovary and oviduct have been clearly established as targets for diethylstilbestrol (DES)-induced dysmorphogenesis. The cellular alterations in the ovary are associated with functional defects--altered steroidgenesis. The epithelial cells of the oviduct no longer maintain appropriate growth controls. Experiments in vitro with the fetal anlage of reproductive tract tissues, the Müllerian duct, have demonstrated that the fetal tract is imprinted by DES at the molecular level and becomes unresponsive to its normal tissue effector, Müllerian Inhibiting Factor. Futhermore the fetal genital tract is capable of oxidatively metabolizing DES along pathways which generate reactive intermediates. This may play a role in the long-term dysdifferentiation of those target tissues, since DES metabolism has been also associated with genetic alteration in model cells in culture (Syrian hamster embryo fibroblasts) including aneuploidy induction and unscheduled DNA synthesis. These occurred under conditions associated with neoplastic transformation of cells in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70065-08 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. S. Korach	Research Endocrinologist	LRDT NIEHS
Others:	J. A. McLachlan	Head, Develop. Endo. and Pharm. Section	LRDT NIEHS
	L. Levy	Research Chemist	LMB NIEHS
	R. P. DiAugustine	Research Chemist	LRDT NIEHS
	J. McKinney	Research Chemist	LMB NIEHS

COOPERATING UNITS *(if any)*
University of Wurzburg
Environmental Chemistry Branch, NIEHS
Medical Foundation of Buffalo
Burroughs Wellcome Research Labs
University of Wisconsin (Madison)
Duke University

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DES, a potent synthetic estrogen and reproductive toxicant has been shown to be extensively metabolized. Some metabolites retain hormonal activity while others are biologically inactive. This assignment of activity is consistent with the receptor binding activity of these compounds. Besides reproductive tract effects some of these metabolites also elicit neuroendocrine effects by suppressing LH secretion. Two groups of metabolites were found to have poor uterotropic activity although they bound very well to the receptor. Some of these compounds show a variety of differences compared to the intracellular responses of DES, including lack of receptor synthesis, poor nuclear translocation, excessive retention of the receptor complex in the nucleus and the inability to stimulate certain tissue responses.

Estrogen stimulation of reproductive tract tissue involves a mechanism which includes binding to a receptor with subsequent activation and localization in the nucleus. Nuclear translocation follows a biomodal temporal pattern consistent with the stimulation of certain tissue responses. Only biologically active compounds induce these receptor events. The two receptor events appear to be occurring in different uterine cell types with uptake in the stroma first followed by the epithelium. Cell cycle kinetic studies of uterine estrogen stimulation show that the second peak occurs at the beginning of S-phase. A major effect of estrogen on uterine cells was to shorten the cell cycle by contracting the G1 phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70067-01 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanism of Steroid Hormone in Sex Organ Development.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. T. Teng	Expert	LRDT NIEHS
Others:	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.1	1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously reported the DNA sequence of a 3.3 kb Eco R1 fragment which contains the entire transcription unit plus 5'- and 3'- flanking regions of the rat seminal vesicle IV gene (SVS IV). The 5'- flanking region of this gene contains a perfect inverted repeat at -117 bp from the CAP site. The potential for forming a true cruciform structure in this region can be demonstrated in supercoiled configuration. Therefore, by S1 nuclease digestion of the supercoiled plasmid SV3.3 (containing SVS IV) we have shown that there is an S1 nuclease sensitive site near the putative cruciform structure. We also detected an S1 nuclease-sensitive site in the 3' flanking region of SVS IV about 800 bp from the termination site of the transcription unit. Recently we studied the S1 nuclease-sensitive and DNAase I hypersensitive sites in the chromatin of the SVS IV gene. We found that the region around -100 to -150 bp was susceptible to the S1 nuclease. DNAase I hypersensitive sites of SVS IV genes were present in seminal vesicle cells in which the gene is expressed and were absent in liver cells in which the gene is repressed. SVS IV gene is undermethylated in seminal vesicle and is highly methylated in liver. We have purified a estrogen responsive secretory protein from mouse uterus through HPLC. This protein is highly glycosylated. Two-dimensional gel electrophoresis indicated this estrogen stimulated protein has molecular weight of 70 KD with pI 6.5-6.8. Antibody against 70K protein has been raised in rabbits. We are in the process of establishing radioimmunoassay for this protein. Two times oligo-dt column purified mouse uterine mRNA from both immature and E2-stimulated animals have been prepared. With the aid of 70K antibody and poly A mRNA, we are able to isolate the cDNA clone which carry the genetic information for 70K protein from mouse uterine cDNA library.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70069-02 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Peptide Growth Factors in Reproduction and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. P. DiAugustine	Research Chemist	LRDT NIEHS
Others:	R. M. Pratt	Head, Exp. Teratogenesis Section	LRDT NIEHS
	R. I. Grove	Staff Fellow	LRDT NIEHS
	O. Hernandez	Research Chemist	LMB NIEHS
	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS
	K. S. Korach	Research Endocrinologist	LRDT NIEHS
	C. T. Teng	Expert	LRDT NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina (Chapel Hill)
Chiron Corporation

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epidermal growth factor (EGF) is a polypeptide capable of stimulating proliferation of a wide range of cell types in vitro. In an attempt to further purify EGF from various commercial sources, we noticed that multiple forms of the polypeptide could be demonstrated by reversed-phase high-performance liquid chromatography. The two major forms, α- and β-EGF, were analyzed to determine any properties unique to that originally reported for EGF. The physicochemical properties of the two peptides were essentially identical but analysis of the two peptides by mass spectrometry and amino acid sequencing indicated the α-EGF had the primary structure originally reported for EGF, and that β-EGF was missing the amino-terminal asparagine. This [Des-Asn^{7}]-EGF was significantly less potent than α-EGF in stimulating proliferation of human embryonic palatal mesenchyme cells. In a second study, ^{125}I-α-EGF was injected intravenously to examine the potential of this peptide to cross the placental barrier in CD-1 mice at different stages of pregnancy (10, 13, and 17 days). At the earliest periods that counts could be detected in embryos (5-10 min) after injection of the trace, embryos were removed, extracted, and the extracts examined by gel filtration; only free ^{125}I was detected. Intact ^{125}I-α-EGF could not be found in plasma or other tissues 5 min after injection. In vitro studies indicate that the peptide can be rapidly degraded to amino acids plus monoiodotyrosine (MIT) by the placenta; some tissues, such as the kidney, can dehalogenate MIT to account for the free ^{125}I that crosses the placenta. The intact peptide, however, exhibits no placental transfer. In a third study, we are exploring the potential role of EGF to mediate estrogen-induced proliferation of uterine epithelial cells. Our present data suggest that mouse uterine epithelial cells in vitro are responsive to EGF, exhibit specific binding sites, and reveal positive cytoplasmic immunolocalization of the polypeptide in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70075-01 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nature and Role of Germ Cell Surface Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Eddy	Head, Gamete Biology Section	LRDT	NIEHS
Others:	D. A. O'Brien	Senior Staff Fellow	LRDT	NIEHS
	I. P. Lee	Pharmacologist	LRDT	NIEHS
	K. Toshimori	Visiting Fellow	LRDT	NIEHS

COOPERATING UNITS *(if any)*
Harvard Medical School Fred Hutchinson Cancer Research Center, Seattle
University of Washington School of Medicine
The Hospital for Sick Children, Toronto, Canada

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Gamete Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gamete cell surface components confer specificity in events critical for processes of reproduction and development. Antibodies can block gamete function and embryogenesis, but the specific molecules involved have not been identified. Monoclonal antibodies are being produced to characterize these molecules, determine their origin and distribution, and define their roles. There are three aspects of the project. (a) One study has examined the appearance of new sperm surface components during epididymal maturation, coincident with sperm gaining the ability to swim and to fertilize. A 54,000 molecular weight antigen being studied is secreted by epithelial cells in the caput epididymidis and attaches specifically to acceptor sites on the plasma membrane of the flagellum. It is cell type and species specific, present in the epithelium after two weeks of age, absent from sperm recovered from the oviduct, lost during in vitro capacitation, but retained on sperm incubated in the presence of mouse or rat epididymal fluid. (b) An additional study has examined sperm surface components that may be involved in the acrosome reaction. Surface molecules are lost during the acrosome reaction as shown by using a monoclonal antibody to monitor antigen loss and a protease activity assay to measure calcium-stimulated acrosomal enzyme release. The protease release was inhibited in sperm exposed to antibody prior to calcium treatment. (c) Another study has examined surface antigens shared by germ cells, teratocarcinoma cells, and embryonic cells. Some of these are high molecular weight glycoproteins and stage-specific expression of particular antigens during spermatogenesis is due to changes in patterns of sialic acid incorporation during post-translational modification of the glycoconjugate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70078-01 LRDT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Stage-Specific Surface Antigens During Mouse Spermatogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. A. O'Brien	Senior Staff Fellow	LRDT	NIEHS
Others:	E. M. Eddy	Head, Gamete Biology Section	LRDT	NIEHS

COOPERATING UNITS *(if any)*

Harvard Medical School
University of Washington School of Medicine

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During spermatogenesis, regional specialization of the plasma membrane occurs resulting in a mature gamete with distinctly polarized functional and biochemical properties. One feature of this differentiative process is the appearance of novel surface constituents in a precise temporal sequence. Both immunological and biochemical techniques have been used to characterize germ cell-specific constituents and monitor membrane assembly. Central to these studies are methods for the purification of germ cells at defined stages of spermatogenesis by enzymatic dissociation of adult or prepuberal testes followed by unit gravity sedimentation. Three areas of research have been pursued: (a) Polyclonal and monoclonal antibodies have been used to characterize macromolecules first appearing on the surface of pachytene spermatocytes, coincident with a period of maximal protein synthesis. These constituents are not shared by most somatic cells and include at least ten proteins, a probable lipid constituent, and large polylactosamine glycoconjugates. Some of these components are retained on sperm, restricted to distinct domains on the cell surface. (b) Conditions for the short term culture of spermatogenic cells are being tested. Earlier meiotic stages from 17-day-old animals exhibit more stringent requirements for in vitro maintenance than later stages from adult mice. Media supplementation with growth factors and hormones has resulted in increased viability of the prepuberal germ cells and should facilitate metabolic studies and the development of in vitro functional assays. (c) Protein synthesis in isolated spermatogenic cells has been examined by 2D PAGE and autoradiography following short term culture with [^{35}S]methionine. Synthetic profiles become more complex throughout meiosis. A number of proteins previously identified as surface antigens are synthesized in a stage-specific manner. Germ cell surface constituents exhibiting both tissue and stage specificity are candidates for further studies exploring cell-cell interactions during spermatogenesis and fertilization.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70080-11 LRDT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: I. P. Lee Pharmacologist LRDT NIEHS

Others: None

COOPERATING UNITS *(if any)*

Laboratory of Environmental Chemistry, NIEHS
Laboratory of Developmental Pharmacology, NICHHD

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies seek to assess mechanisms by which environmental chemicals modify gene expression of male reproductive organs. The following studies are ongoing: (1) the mechanism by which DNA modifiers increase the TCDD-induced P_1-450 mRNA of prostate glands, and (2) the study of possible mechanisms for potentiation of TCDD-activated prostatic P_1-450 gene by DNA modifying agents. This project is being incorporated into project number Z01 ES 70085-07 LRDT.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70085-07 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developoment of In Vitro Models For Assessing Reproductive Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I. P. Lee	Pharmacologist	LRDT	NIEHS
Others:	D. W. Nebert	Chief	DP	NICHHD
	C. Parker	Analytical Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Environmental Chemistry, NIEHS
Laboratory of Developmental Pharmacology, NICHHD

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Gamete Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies seek to assess the effects of environmental agents on spermatogenesis, sperm maturation, functions of accessory organs, and male reproductive capacity. Mechanisms of toxicity are studied, and new approaches to toxicity testing are proposed and validated in order to extrapolate more reliably from laboratory animals to man and to improve our ability to analyze risk. The following studies are in process: (1) to define the biochemical nature of the molecules of epididymal monoclonal antibodies, the site of action, and the origin of sperm surface glycoproteins; (2) the study of the functional role of the sperm surface molecules and the identification of environmental chemicals which perturb these molecular processes; and (3) mechanisms by which environmental chemicals modify gene expressions of male reproductive organs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 ES 70090-01 LRDT

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine and Neurochemical Regulation of Gonadal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Head, Reprod. Neuroendocrinology Section	LRDT	NIEHS
Others:	C. A. Johnston	Staff Fellow	LRDT	NIEHS
	M. M. Valenca	Visiting Fellow	LRDT	NIEHS
	E. Spinedi	Visiting Associate	LRDT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.1	2.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiments carried out by the Reproductive Neuroendocrinology Section are focused on the cellular and subcellular mechanisms regulating the release of luteinizing hormone-releasing hormone (LHRH) and other hypothalamic peptides that participate in the modulation of pituitary hormone release. Specific studies are designed to elucidate the role of monoaminergic neurotransmitters in the release of LHRH from nerve terminals, the nature of the specific aminergic receptors involved in the neuronal activation that precedes LHRH release, the clarification of the post-receptor events that participate in the peptide-release process, the involvement of arachidonate metabolites in amplifying the response to key neurotransmitters, and the additional role played by other intracellular messengers such as Ca^{+2} and other putative intracellular messengers derived from the metabolism of membrane phospholipids. Other parts of the project are directed to perform an in-depth analysis in vivo of the changes in monoamine turnover and metabolism in discrete brain nuclei that are known to be involved in regulation of gonadal function. Different experimental paradigms are employed, to re-create situations calling for an enhanced (or altered) function of the hypothalamic-pituitary-gonadal axis, such as steroid-feedback manipulations, pregnancy, lactation, estrous cycle, stress, ablation of selected endocrine glands or brain areas, etc. Finally, a group of experiments are directed to evaluate the mechanisms underlying the effects on the reproductive sphere of neonatal neurotoxin treatment, as well as the developmental changes and the role of steroids on certain sexually dimorphic patterns of gonadotropin secretion. The results are integrated to provide a comprehensive hypothesis of the complex, multi-level regulatory mechanism modulating gonadal function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70092-01 LRDT

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Molecular Mechanisms Mediating Peptide Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Head, Reprod. Neuroendocrinology Section	LRDT NIEHS
Others:	E. Spinedi	Visiting Associate	LRDT NIEHS
	C. A. Johnston	Staff Fellow	LRDT NIEHS
	M. M. Valenca	Visiting Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of the cellular and molecular mechanisms mediating peptide hormone action constitute an important component of the research efforts of the Reproductive Neuroendocrinology Section. The close interrelationships mediating neuroendocrine responses within the hypothalamic-pituitary-gonadal systems offer an excellent opportunity to analyze some unique characteristics of peptide-peptide, peptide-amine, and peptide-amine-steroid interactions. Studies using pituitary cell cultures are directed to evaluate the precise mechanisms through which peptidergic or aminergic secretagogues enhance or suppress peptide hormone release. Protocols are designed to evaluate characteristics of hormone-receptor interactions, post-receptor as well as transmembrane events involved in the hormone-release process, and definition of the specific intracellular messengers transducing the action of key hypothalamic peptides involved in pituitary hormone release. Other studies are designed to explore the intriguing peptide-amine interactions (such as serotonin-AVP) previously reported by our group, to better understand the nature and physiological significance of those interactions.

At the testicular level, studies are designed to determine the intratesticular effects of LHRH-analogs, known to adversely affect both the endocrine and the gametogenic functions of the testis. Since some of these analogs are presently being tested for use in human contraception, an understanding of their site(s) and mechanisms of action is of obvious significance. The interaction of these LHRH-A with intrinsic peptidergic systems within the testis, such as the proopiomelanocortin-derived peptides, is also being explored. The results may provide very significant advances to our knowledge of paracrine and/or autocrine effects of gonadal peptides.

COMPARATIVE MEDICINE BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22102-03 CMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of a Coronavirus from Rabbits

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	J. D. Small	Head, Diagnostic Laboratory	CMB, NIEHS
Others:	M. E. Clements	Bio. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch, TRTP, NIEHS, NIH (Dr. Thompson); Division of Comparative Medicine, Johns Hopkins School of Medicine (Drs. J. Strandberg and L. Aurelian)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to study the pathogenesis of rabbit coronavirus (RbCV), the rabbit's physiologic response to this virus, and the relatedness of RbCV to other members of the Coronaviridae. Pleural effusion disease (PED) virus and RbCV have been shown to induce similar clinical signs and lesions. Each virus induces antibody which partially neutralizes both viruses. Blood from rabbits given RbCV exhibited decreased platelet counts, RBCs, hematocrits, and hemoglobin levels. Leukopenia followed by leukocytosis with a left shift was seen. Serum creatine kinase (CK) levels increased as much as 217 times pre-inoculation levels on day 4 post infection. With the elevated CK, lipemia was common. Prothrombin time increased from 9.1 seconds to greater than 150 seconds 4 days following infection.

Assessment of myocardial damage using ECGs and creatine kinase isozymes are beginning. Likewise, further examination of coagulation factors, activated partial thromboplastin time, prothrombin time, thrombin time and fibrinogen levels are planned. Antibodies to other coronaviruses are being produced for use in studying the relatedness of RbCV to other Coronaviridae.

The significance of this work lies in the ability to study a viral disease with a cardiotropism in an animal of sufficient but manageable size to allow sequential clinical and physiological observations. The damage to the rabbit heart by RbCV has a corollary in the human heart with the Coxsackie viruses, Mycoplasma pneumoniae, influenza virus, Herpes zoster, and possibly other infectious agents.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22104-01 CMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of the Mouse Bioassay Test for Detecting Estrogenic Activity in Feed

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Thigpen	Microbiologist	CMB, NIEHS
Others:	C. B. Richter	Vet. Med. Ofcr.	CMB, NIEHS
	L. A. Li	Biostatistician	BRAP, NIEHS
	J. K. Haseman	Biostatistician	BRAP, NIEHS
	C. W. Jameson	Chemist	TRTP, NIEHS

COOPERATING UNITS *(if any)*
Animal Husbandry

LAB/BRANCH
Comparative Medicine Branch

SECTION
Quality Assurance Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.8	.3	.5

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
 - ☐ (a1) Minors
 - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project were to evaluate and make recommendations for improving the mouse bioassay (NIH, Std. No. 2C, 12-8-72) for detecting estrogenic activity in animal diets. The test diets from 9 of 9 bioassays were reported to be non-estrogenic by a contract testing laboratory. When results were analyzed, 3 of 9 bioassays were determined to be invalid. The mean uterine weight to the terminal body weight ratio for the positive control mice was not 1.5 times as great as that of the negative control mive, as required for a valid bioassay. Uterine weights for the negative control mice of the 3 invalid bioassays were significantly heavier (P less than .01) than the uterine weights for the negative control mice of the 6 valid bioassays. These higher uterine weights in some of the negative control mice may have been due to endogenous hormone activity, probably as a result of failure to control test animal age within the recommended range (14-17 days). Uterine weight increases caused by hormonal activity may occur as early as 22 days of age in some female mice; hence, bioassay studies should be concluded before uterine weight increases due to endogenous hormone begin. To establish the lower limit of useable age, female mice were weaned at 14, 16, 18, or 20 days of age and body growth curves determined. Mice weaned at 14 days and weighed 2 days later lost weight; however, by 24 days of age, they were indistinguishable from mice weaned at 16, 18, or 20 days. Female mice weaned at 16 days of age were chosen to determine the body : uterine weight ratio growth curves. These females showed increases with minor variations in weights from 16-22 days of age. After 22 days of age rapid uterine growth occurred in some mice resulting in large variations in uterine weights from 22-32 days. These results suggest that data collected beyond the 22 day age are misleading in terms of the bioassay, and that only carefully controlled age ranges of weanling mice are suitable for the bioassay.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22105-01 CMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effects of Trichlorfon on the Immune System of the Mouse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. D. Small	Vet. Med. Ofcr.	CMB, NIEHS
Others:	K. R. Brodie	Biological Aid	CMB, NIEHS
	M. E. Clements	Bio. Lab. Tech.	CMB, NIEHS
	E. H. Lebetkin	Bio. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS (if any)
Systemic Toxicology Branch, TRTP, NIEHS

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.1	.1

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
 - ☐ (a1) Minors
 - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Trichlorfon, o,o-dimethyl 2,2,2-trichloro-1-hydroxyethyl-phosphonate, an organophosphate pesticide and vermifuge has been used to treat laboratory mice, rats, and hamsters infected with the pinworm, Syphacia obvelata. A concern was the effect of this drug on the animal's immune system. The immunotoxicity of this drug was examined in B6C3F1 mice. IgM antibody production against sheep red blood cells, delayed hypersensitivity to keyhole limpet hemocyanin, and the lymphoproliferative response to E. coli lipopolysaccharide were used to assay B cell function. T cell function was measured by responses to concanvalin A and phytohemagglutinin. No significant effects were observed using the above criteria. A statistically significant increase in liver and kidney but not spleen weight and organ to body weight ratio was observed. These weight differences are thought to be due to the decreased water intake in the drug treated group.

The significance of this project lies in the fact that trichlorfon kills the parasite and is more efficacious than the more commonly used alternative, piperazine, which does not kill the parasite. This data suggests that the use of trichlorfon in mice will not compromise the host's immune system. The increased liver and kidney weights will be examined in a second group of mice.

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21001-04 CTEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Chemical Nephrotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William M. Kluwe	Acting Chief	TRTP/CTEB	NIEHS
Others: Deepak K. Agarwal	Visiting Fellow	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3/4	1/4	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Time- and dose-dependent effects of selected nephrotoxic agents on the ultrastructure and functional and biochemical status of target and non-target cells in the kidney are evaluated to study basic mechanisms of injury to various renal cell populations. Comparisons are made between chemical structures and the types of subcellular lesions induced, or the target cells affected, to elucidate common pathophysiological sequences of chemically-induced renal cell injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21030-02 CTEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemically-induced and Spontaneous Mononuclear Cell Leukemia in Fischer 344 Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. French	Physiologist	TRTP/CTEB	NIEHS
Others:	Michael P. Dieter	Physiologist	TRTP/CTEB	NIEHS
	Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS
	Ralph Wilson	Bio. Lab. Tech.	TRTP/CPB	NIEHS

COOPERATING UNITS *(if any)*
Systemic Toxicology Branch/TRTP/NIEHS
Chemical Pathology Branch/TRTP/NIEHS
Program Resources Branch/TRTP/NIEHS

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NTP chronic toxicity test results indicate a high incidence of rat mononuclear cell leukemia (MNCL) in both control and treated F344 rats. It is difficult to determine if chemical induction of MNCL has occurred in a chronic test because of the high incidence of spontaneous MNCL. Responses to chemical treatment have included statistically significant increases or decreases in MNCL as well as positive or negative trends with respect to dose. A transplant model for MNCL has been characterized in terms of its clinical presentation, gross pathology, cell morphology and biochemistry, histopathology, and clinical hematology and chemistry. After subcutaneous inoculation of 2 X 10E7 viable leukemic cells into rats, clinical symptoms, morbidity and mortality due to MNCL occur after 100 days (95 to 105 days). Clinical symptoms are usually present after 90 days. However, with this size inoculum, splenomegaly and leukocyte count increase start to occur after 65 days. The FY 1985 objective of this research project is to develop a short-term test model for assessing the effect of chemical treatment on the expression of transplanted mononuclear cell leukemia in F344 male rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21061-02 CTEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between Chemical Dose and Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William M. Kluwe	Acting Chief	TRTP/CTEB	NIEHS
Others: Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS
Jerry Hardisty	Pathologist	Environmental Pathology Labs, Raleigh, NC	

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5/8	1/2	1/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several organohalide compounds are metabolized to reactive intermediates presumed to be the ultimately toxic molecules. The reactive metabolites are detoxified by reacting with tissue non-protein sulfhydryls (NPS), and acute toxicity occurs only when NPS have been depleted below a critical level. Upon prolonged chemical exposure, however, a dynamic state exists between chemical metabolism, NPS depletion, NPS synthesis, and lesion development. Whether or not the same relationship exists between tissue NPS concentrations and the development of lesions in a chronic exposure situation as in an acute one is being evaluated. Also, the organ-specificity and species-specificity of these phenomena are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21062-02 CTEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Phthalate Ester Toxicities in Mammalian Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	William M. Kluwe	Acting Chief	TRTP/CTEB	NIEHS
Others:	Deepak K. Agarwal	Visiting Fellow	TRTP/CTEB	NIEHS
	James C. Lamb, IV	Biologist	TRTP/STB	NIEHS
	Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS
	Scott Eustis	Pathologist	TRTP/CPB	NIEHS
	Ronald L. Melnick	Biochemist	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1-3/8	7/8	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phthalate esters are plasticizers incorporated into nearly all plastic materials. The biochemical and ultrastructural effects of di(2-ethylhexyl)phthalate (DEHP) and related chemicals are being studied in order to assess potential mechanisms of phthalate ester toxicity.

Since DEHP and other phthalates are also male chemosterilants and teratogenic in mice, studies are being conducted to determine the role of zinc in the pathophysiology of these reproductive effects and to discern no-observed toxic effect levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21063-02 CTEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tests for the Detection and Monitoring of Chemical-Induced Pulmonary Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rajendra S. Chhabra	Supv. Pharamcologist	TRTP/CTEB	NIEHS
Others:	Gary E.R. Hook	Research Chemist	LPFT	NIEHS
	Paul Nettesheim	Chief	LPFT	NIEHS
	Kimeri D. Collins	Bio. Lab. Tech.	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

Biochemical Pathology Group, LPFT

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to develop procedures for (1) determining the potential of agents in causing lung damage and (2) detecting pulmonary injury and monitoring that injury as it progresses towards or away from the disease state. Methods for the estimation of injury potential involves the use of purified cell populations isolated from the lungs and the reaction of those cells to toxic agents. Methods for the detection of pulmonary injury involve the use of pulmonary lavage effluents and the identification of markers of cellular injury. Two cell populations have been isolated from the lungs of rabbits. Clara cells from the airway epithelium have been isolated and purified to as much as 95% with 97% viability as measured by exclusion of trypan blue. Type II cells have also been isolated and purified to as much as 85% with 88% viability. Incubation of clara cells and Type II cells with naphthalene revealed that Type II cells were considerable more sensitive to the toxicity of this agent than Clara cells. Incubation of cells with naphthalene (1mM) for a period of one hour resulted in a 20% loss in viability of the Type II cells whereas Clara cells and alveolar macrophages were unaffected. Lavage effluents from the lungs of rats treated with silica by intratracheal injection showed elevated levels of some biochemical parameters. Increases were all dose- and time-dependent. These investigations indicate that isolated cells could be used to test the cellular toxicity of chemical agents and that lavage effluents from the lungs of animals exposed to toxic agents may be used for the detection of pulomonary lung injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21076-01 CTEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Biochemistry Studies on Chemical Selected for Evaluation by NTP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael P. Dieter	Physiologist	TRTP/CTEB	NIEHS
Others:	Ralph Wilson	Bio. Lab. Tech.	TRTP/CPB	NIEHS
	Gary A. Boorman	Pathologist	TRTP/CPB	NIEHS
	Michael I. Luster	Immunologist	TRTP/STB	NIEHS
	Linda S. Birnbaum	Pharmacologist	TRTP/STB	NIEHS
	John E. French	Physiologist	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*
Systemic Toxicology Branch, TRTP
Chemical Pathology Branch, TRTP
Program Resources Branch, TRTP

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of immunotoxic chemicals on the intermediary metabolism of specific reticuloendothelial cells (macrophages and T-lymphocytes) depressed in aged rats, correlated with the known deficit in cell-mediated immunity that occurs in aging mammals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21077-01 CTEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Effects of Methyldopa on the Reproductive System of F344/N Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	June K. Dunnick	Toxicologist	TRTP/CTEB	NIEHS
Others:	Leroy B. Hall	Pathologist	TRTP/CPB	NIEHS
	James C. Lamb, IV	Research Biologist	TRTP/STB	NIEHS
	Martha Harris	Technical Supervisor	TRTP/CPB	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch, TRTP, NIEHS
Systemic Toxicology Branch, TRTP, NIEHS

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	0.4	2.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methyldopa (L-α-methyl-3,4-dihydroxyphenylalanine) is used for the treatment of hypertension, and is listed as one of the top 25 drugs in the United States, with over 22 million prescriptions written per year. Side effects of methyldopa have included sexual dysfunction in humans, and in the NTP subchronic studies testes and uterus were reported to be target tissues in the Fischer 344/N rats.

This NTP intramural research project is designed to determine the potential toxic effects of methyldopa on the reproductive system of male Fischer 344/N rats. Methyldopa was administered for eight weeks at doses of 0, 50, 100, 200 and 400 mg/kg. Treated male rats were mated to untreated female rats. Male rats were evaluated for histopathologic abnormalities in the reproductive organs and sperm abnormalities. The results of the mating trial, histopathologic analysis, and sperm analysis are currently being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21078-01 CTEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioavailability and Toxicity Studies of Microencapsulated Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Others:	C.W. Jameson	Chemist	TRTP/PRB	NIEHS
	T. Goehl	Chemist	TRTP/PRB	NIEHS
	J.H. Mennear	Pharmacologist	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

Midwest Research Institute, Kansas City, MO
Program Resources Branch, TRTP

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Trichloroethylene (TCE) and 2,6-xylidine, two volatile chemicals, have been separately encapsulated in gelatin-sorbitol microcapsules. These formulations have been shown to provide sufficient stability to the chemicals so that they may be useful in dosed-feed toxicology studies. The objectives of this project are to compare the rates and extents of absorption of neat and microencapsulated chemicals in rats and mice, and to evaluate the feasibility of using microencapsulation as a means of incorporating unstable test chemicals into rodent feed for toxicology studies. The rates and extents of absorption of TCE, prepared either as a suspension of microencapsulated TCE in corn oil or as a solution of neat TCE in corn oil, administered by gavage to male Fischer 344 rats have been studied. Similar studies in B6C3F$_1$ mice are being developed. 14-Day dosed feed toxicity studies of microencapsulated TCE in rats and mice are intended for the fourth quarter of FY 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21079-01 CTEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Di(2-ethylhexyl)phthalate Hepatotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Others:	William M. Kluwe	Pharmacologist	TRTP/CTEB	NIEHS
	Deepak K. Agarwal	Visiting Fellow	TRTP/CTEB	NIEHS
	K. Tomaszewski	Visiting Fellow	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a rodent bioassay conducted by the National Toxicology Program, di(2-ethylhexyl)phthalate (DEHP) was found to be carcinogenic for the liver in B6C3F$_1$ mice and F344 rats. Since DEHP also causes peroxisome proliferaton, it has been suggested that the carcinogenicity of this chemical may be related to excessive peroxisomal production of H_2O_2. It is the objective of this project to examine the changes in H_2O_2 production from peroxisomal fatty acyl-CoA oxidation in livers of rats and mice treated with DEHP. Further assessment of an involvement of reactive intermediates of oxygen reduction in DEHP induced hepatotoxicity will be made from measurements of (a) activities of enzymes that eliminate toxic oxygen products (catalase, superoxide dismutase, glutathione peroxidase), (b) lipid peroxidation, and (c) superoxide anion radical production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30100-05 CTEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toxic Effects of 1,2-Dibromo-3-chloropropane on the Urogenital System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William M. Kluwe	Acting Chief	TRTP/CTEB	NIEHS
Others:	James C. Lamb, IV	Biologist	TRTP/STB	NIEHS
	Deepak K. Agarwal	Visiting Fellow	TRTP/CTEB	NIEHS
	Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS
	Bhola N. Gupta	Pathologist	TRTP/CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3/4	1/4	1/2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 - ☐ (a1) Minors
 - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acute and subchronic toxic effects of the pesticide 1,2-dibromo-3-chloropropane (DBCP) and structurally-related compounds are studied from functional and mechanistic viewpoints. A reported chemo-sterilant in humans, DBCP is no longer manufactured in the U.S., but its presence in ground water and on edible imports and its illegal bulk transport into certain areas of the U.S. require its further toxicological characterization. Effects of DBCP on hepatic, renal, and reproductive functions and development are evaluated at several dose levels, after various treatment regimens and under differing conditions such as age, chemical or physical stress and the like.

Comparative toxicities of DBCP and its metabolites are being evaluated to ascertain the toxic chemical moiety and to predict whether structurally similar chemicals would produce the same toxic effects as does DBCP.

CELLULAR AND GENETIC TOXICOLOGY BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21012-03 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organ and Species Specificity of Chemical Carcinogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Langenbach	Microbiologist	CGTB	NIEHS
Others:	Nancy Stammer	Biological Lab Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*

S. Nesnow, EPA

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An in vitro approach for studying organ and species differences in the activation of chemical carcinogens has been previously developed by the Principal Investigator (PI). Both intact cells and cell homogenates have been used for metabolic activation although intact primary cells are most frequently used as they best simulate in vivo metabolism. To assess biological activity, the multiple genetic endpoints of toxicity, mutation and SCE induction in V79 cells and reversion of S. typhimurium are used. In addition, HPLC analysis of metabolites formed by primary cells from different organs and species have been conducted. Most of the work during the past year have focused on differences in liver and bladder cell activation/metabolism from rat, dog, and bovine. One outcome of this research has been the observed species difference in the relative capability of these organs to activate carcinogenic aromatic amines. Results from another study have indicated the differences between rat and hamster liver cells in the activation of nitrosamines and aromatic amines and the relative sensitivities of the genetic endpoints listed above to the activated intermediates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NO.

Z01 ES 21013-03 CGTB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Gene Toxic/Carciongenic Events in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. R. Boone	Senior Staff Fellow	CGTB	NIEHS
Others:	R. W. Tennant	Supervisory Microbiologist	CGTB	NIEHS
	P. L. Glover	Bio. Lab. Tech.	CGTB	NIEHS
	C. L. Innes	Bio. Lab. Tech.	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Wen K. Yang — Biology Division, ORNL (Contract YO1-ES-10061)

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.9	1.2	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the role of endogenous ecotropic provirus in radiation induced and spontaneous hematopoietic neoplasms of the RFM/Un mouse. Our results indicate that somatically acquired provirus is integrated at novel sites in most reticulum cell sarcomas, and some radiation induced myeloid leukemias and thymic lymphomas. Detailed analysis of recombinant DNA clones of two somatically acquired proviruses in myeloid leukemia indicate that they are integrated into unique DNA regions that may be rearranged in independent tumors. These sequences may represent a cellular oncogene or other critical gene involved in specific neoplastic phenotype.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21014-03 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Mathematical Modeling of DNA Repair and Recombination in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Resnick	Research Geneticist	CGTB	NIEHS
Others:	T. Darden	Staff Fellow	BRAP	NIEHS
	J. Nitiss	Biological Lab. Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Genetic recombination is an essential feature of normal meiosis and during repair of various types of DNA damage. As part of a program to understand the mechanisms of recombination and its genetic control, the timing and the location of recombination events are being evaluated by applying mathematical simulation procedures to biochemical data. DNA lesions can be used as markers in exchange processes where the lesions can be identified using enzymes which will nick the DNA, and thus reduce the size of the DNA. If recombination occurs, lesions induced in parental strands of DNA can become associated with newly snthesized DNA; therefore, as a reuslt of recombination events, newly synthesized DNA can become sensitive to nicking enzymes. Using this approach, we have been able to predict the detectibility of exchanges by mathematical modeling, knowing the average number of lesions per parental molecule, and to relate exchanges to models for recombination. Biological results from E. coli, yeast, and mammalian cells are being evaluated using the mathematical simulation.

In rad52 strains of yeast which are lacking in DNA repair, meiosis is defective. Cells accumulate rare single-strand interruptions in their chromosomal DNA during meiosis which are likely to be associated with recombination in wild type strains. To identify their frequencies in individual chromosomes and their distributions, we have utilized small probes to specific chromosomes. The chromosomal DNA is separated according to size on sucrose gradients and individual fractions are hybridized with the probe. We have utilized mathematical modeling to analyze the best positioning for a probe relative to the end of the chromosome in order to detect rare interruptions. The actual probing data were analyzed mathematically in terms of various models. The distribution of SSIs is clearly nonrandom which would indicate specificity of recombination sites in yeast.

GPO 904-917

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymes Involved in DNA Repair and Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Michael Resnick	Research Geneticist	CGTB	NIEHS
Other:	Terry Chow	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The RAD52 gene in Saccharomyces cerevisiae controls the repair of ionizing radiation-induced DNA double-strand breaks, radiation-induced spontaneous mitotic recombination, and recombination during meiosis. Utilizing an antibody raised against a Neurospora crassa deoxyribonuclease, we observed that in logarithmically growing wild type yeast strains, approximately 80% of Mg^{++} dependent pH 8 single-strand deoxyribonuclease is antibody precipitable. As cells enter stationary phase the antibody-precipitable nuclease decreases to an undetectable level as does cross-reacting material. The antibody precipitable nuclease activity increases five to ten times during meiosis during the period of DNA synthesis and recombination, and decreases at the end of meiosis. No activity is observed in rad50 or rad52 mutants; these results are correlated with the lack of recombination in these mutants. The nuclease that is detected with the antibody has been purified nearly 1000-fold and has a molecular weight of 70,000. It is a single-strand endo-exonuclease which also exhibits double-strand nuclease activity.

These observations on the biochemical activity of the enzyme, its appearance during meiosis, and the genetic control by the RAD52 gene, implicate this exo-endonuclease in repair processes, spontaneous and damage-induced mitotic recombination, and normal meiotic recombination.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21028-03 CGTB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Salmonella Plate Test

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others: Dennis Pagano	Research Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of studies are underway which are designed to improve our understanding of the dynamics of the Ames Salmonella microsome test, to make improvements in the standard protocols, to make the test easier to run, and/or to make the test results more readily interpretable. These studies include the effects of substances which may be present in environmental mixtures and which will interfere with a positive mutagenic response in the apparent absence of cell toxicity; characterization of the new tester strains, TA97 and TA102; the effect of prolonged storage of mutagen solutions used for positive controls; and the effect of the commonly-used solvent, DMSO, on the Salmonella tester strains.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21045-02 CGTB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of SPO11, a Gene Required for the Early Events of Meiosis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C.N. Giroux Senior Staff Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to identify and analyze the cellular functions which are required specifically for meiosis in the yeast, Saccharomyces cerevisiae. In particular, we are focusing on the analysis of the SPO11 gene of yeast which is required for recombination and proper chromosome segregation during meiosis. A general system has been developed to isolate meiosis specific genes of yeast for which mutants are available. Using this system, the $SPO11^{+}$ wild type gene has been isolated following transformation and complementation of a spo11-1 mutant with a total genome clone bank. The function of the cloned gene has been examined during a detailed analysis of the complementation of the spo11-1 mutant by the isolated $SPO11^{+}$ gene. The structure of the cloned gene has been partially determined by restriction enzyme analysis. The structure of the cloned gene will be further analyzed by fine structure restriction analysis and by subcloning. The function of the cloned gene will be further characterized by mutagenesis of the cloned DNA and by substitution of the chromosomal $SPO11^{+}$ gene by in vitro engineered constructions. We will try to identify the gene product of the $SPO11^{+}$ gene by expression of the cloned gene in an E. coli system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21048-01 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of a Molecular System to Study Mutagenesis in Yeast

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: C.N. Giroux Senior Staff Fellow CGTB NIEHS

COOPERATING UNITS (if any)
Dr. Bernard Kunz, Biology Department, York University, Toronto, Ontario

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this project is to investigate the mechanisms whereby genetic information is transmitted to progeny somatic cells with fidelity: how mutagenesis occurs and what mechanisms the cell employs to avoid mutation. Using a combination of classical genetic and recombinant DNA techniques, we are constructing a model system to examine the molecular basis of mutagenesis in the yeast, Saccharomyces cerevisiae. A yeast tester strain is under construction which will enable the mutagenesis of a cloned SUP4 tRNA suppressor gene to be assayed by direct genetic selection and DNA sequence analysis. In addition, the tester strain will allow the role in mutagenesis of the three genetically defined repair pathways of yeast to be examined using the same target plasmid.

PHS 6040 (Rev. 1/94) GPO 904 917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21049-02 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
DNA Synthesis and Metabolism During Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. A. Resnick	Research Geneticist	CGTB	NIEHS
Others:	T. Chow	Visiting Fellow	CGTB	NIEHS
	J. Nitiss	Biological Laboratory Technician	CGTB	NIEHS
	J. Westmoreland	Biological Laboratory Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*
Dr. Akio Sugino, University of Georgia, Athens, GA, and LG, NIEHS

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Unique DNA metabolic activities have been implicated during meiosis and following exposure of mitotic cells to DNA damaging agents. To understand the processes involved, it is important to examine the enzymes that are presumed to be responsible. We have characterized both the DNA and the DNA metabolic enzymes at various times in meiosis in wild type and repair-deficient cells of yeast. DNA polymerases I and II increase by approximately two and three times, respectively, during meiosis shortly before the time of meiotic DNA synthesis and recombination. Low levels of pH 8.0 single-strand deoxyribonuclease activity are observed in cells prior to the beginning of meiosis. The activity increases during meiosis beginning around the time of premeiotic DNA synthesis. Of particular interest is a nuclease under the control of the RAD52 gene. It increases five to ten-fold in RAD^+ cells and decreases toward the end of meiosis. No activity is detected in rad52 cells. It therefore appears that there is a coordinated increase in enzyme systems involved in meiotic DNA synthesis and recombination. We are also investigating signals which might be involved in meiotic DNA metabolic events. Although there is very little methylation of DNA in yeast, it appears that there are sequences which become methylated or lose methylation during meiosis. The timing and function of these are being pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

NUMBER

Z01 ES 21051-01 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.:	James M. Mason, Ph.D.	Geneticist	CGTB	NIEHS
Others:	Akihiko H. Yamamoto, Ph.D.	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*
Department of Genetics, University of California, Davis

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutagen-sensitive mutants defective in DNA repair mechanisms have been collected in Drosophila melanogaster and characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. The tests used in the initial characterization of these mutants include genetic and cytogenetic mapping, complementation analysis, tests for sensitivity to unrelated mutagens, and tests for pleiotropic effects on related functions such as recombination. A genetic fine structure map of the mei-41 region has been constructed using several independently isolated alleles. This map confirms the large size of mei-41 found during mutational analysis. The mei-41 locus is estimated to cover approximately 300 kilobase pairs of DNA.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21052-02 CGTB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of Xenobiotics to Mutagens Using Non-hepatic Microsomal Enzyme Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Z. Matijasevic	Visiting Fellow	CGTB	NIEHS
	D. Pagano	Research Microbiologist	CGTB	NIEHS
	T. Eling	Head, Prostaglandin Group	LFPT	NIEHS
	I. Lee	Pharmacologist	LDRT	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pulmonary Function and Toxicology
Laboratory of Developmental and Reproductive Toxicology

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Xenobiotic chemicals can be metabolized by organs and tissues other than the liver. Additionally, the prostaglandin endoperoxide synthetase (PES) system is found in a number of organs and is not dependent on cytochrome P-450. The metabolism of polycyclic aromatic hydrocarbons, aromatic amines, and other chemicals to mutagens in Salmonella tester strains was studied using PES and, also, microsomal preparations from rat lungs, testes, and prostate, in addition to liver.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21053-01 CGTB

PERIOD COVERED
October 1, 1983 to September 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Control of Mutation in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	James M. Mason	Geneticist	CGTB	NIEHS
Others:	Larry Champion	Biological Lab Technician	CGTB	NIEHS
	Barry Margolin	Mathematical Statistician	BRAP	NIEHS

COOPERATING UNITS *(if any)*
Department of Biological Science, Purdue University
Department of Genetics, University of California at Davis

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutation rates are under genetic control. In bacteria and yeast, the frequency of induced mutations can be either increased or decreased by blocking one or another pathway of DNA repair. This project is designed to determine the relationship between DNA repair and mutagenesis in Drosophila melanogaster. Three approaches are being taken: (1) A mutant which increases the mutation frequency (a mutator) has been identified, mapped, and characterized. This mutator blocks repair of chromosome breaks specifically in oocytes allowing a previously undescribed repair process to be observed. In this process broken chromosomes are "healed", allowing the recovery of terminal deletions. (2) The interaction of DNA repair-defective mutants and transposable elements has been observed in double mutant combinations. None of the repair-defective mutants examined to date influence the rates of transposon-induced mutation or recombination, although mutants at the mei-41 locus prevent the transmission of transposon-bearing chromosomes. (3) Two statistical analyses have been compared for their applicability to mutagenicity experiments that produce binomial responses from a control group and a single experimental group.

HHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21054-01 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
DNA Damage and Repair in Centromeres of Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: M. A. Resnick	Research Geneticist	CGTB	NIEHS
Others: J. Westmoreland	Biological Lab. Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*
Dr. Kerry Bloom, Assistant Professor, University of North Carolina, Chapel Hill

LAB/BRANCH
Cellular and Genetic Toxicology Branch

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The induction of DNA damage in chromosomal DNA would be expected to be dependent on the total structure of chromosomes within cells. Protein associations, folding and extent of superhelicity of DNA within chromosomes could influence the induction and distribution of damage. Because of the role that centromeres play in chromosome segregation, we have begun to examine the distribution of damage in the centromeric region of cellular DNA. Excision-defective strains of yeast are irradiated with UV, the DNA is gently extracted and treated with UV-endonuclease to produce nicks next to pyrimidine dimer sites. The chromosomal DNA is then probed with a probe specific for the centromere of chromosome III. At present it appears that the centromeric region is resistant to dimer induction. At high doses, however, dimers are detected; they appear in the AT rich regions of the centromere although they would be expected in other parts of the probed region.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21058-02 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Dosimetry of Ethylmethane Sulfonate in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Z. Matijasevic	Visiting Fellow	CGTB	NIEHS
Others: E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Biology and Microbial Genetics, University of Zagreb, Yugoslavia

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mutagenic activity of ethylmethane sulfonate (EMS) as a function of its DNA alkylating ability has been studied in Salmonella typhimurium. The mutagenic activity of EMS in the base-pair substitution strain G-46 and its repair deficient derivatives (TA1950, [uvrB]; TA92, [pKM101]; TA2410,[uvrB, pKM101]) were compared. Mutation frequencies increased as a function of total DNA alkylation. Ethylation levels were equivalent in wild type and uvrB⁻ cells, but the efficiency of induction of mutations (mutants/adduct) was different between the two cell types. This will provide a reference for the effects of the various repair deficiencies on EMS-induced mutagenesis and provide data to allow us to relate treated dose to delivered dose to mutagenic response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 ES 60102-06 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	D. Pagano	Research Microbiologist	CGTB	NIEHS
	Z. Matijasevic	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Reproductive and Developmental Toxicology

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chemicals of interest have been tested for mutagenicity using a number of Salmonella tester strains. The chemicals studied this year, or being studied, were diethylstilbestrol (DES), sodium bisulfite (SB), sodium azide, halothane, a series of nitrosamines, and pyrene. DES has yielded negative results in all mutation assay systems used. SB, an antioxidant, has been shown to be mutagenic at low pH, and the mutagenicity was bacterial strain-specific. Sodium azide, a known mutagen, and widely used as a positive control in the Salmonella assay, can form a volatile mutagen. This study was done in order to show if production of the volatile mutagen can interfere with the results on plates not containing azide but in the same incubator as plates with azide. The benzo(a)pyrene analog, pyrene, which up to now has given inconsistent mutagenicity data, was tested with a newly constructed Salmonella strain TA97 as well as some of the others.

PHS 6010 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

NUMBER Z01 ES 60122-05 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of DNA Repair in Yeast and Their Role in Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Resnick	Research Geneticist	CGTB	NIEHS
Others:	John Nitiss	Biological Lab. Technician	CGTB	NIEHS
	Jim Westmoreland	Biological Lab. Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*
J. C. Game, University of California, Berkeley, Department of Genetics

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
1.5	0.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA repair mechanisms which have been identified in mitotically growing cells of the yeast Saccharomyces cerevisiae are being examined for a) their ability to protect cells undergoing meiosis from DNA-damaging agents, and b) the role of the corresponding genes in normal meiosis. We have developed sucrose gradient techniques to examine repair in mitotic or meiotic cells after low doses of both UV and ionizing radiation and to follow changes in meiotic DNA during meiosis in various rad mutants. Methods have been developed for characterizing rare interruptions in chromosomal DNA as well as locating sites of interruptions in specific chromosomes.

There appears to be only one system for excision-repair throughout meiosis; it is controlled by the rad1 gene product. Cells can tolerate approximately 1500 pyrimidine dimers during early stages of meiosis due to an ability to synthesize DNA past dimers; as cells proceed through meiosis the damage has a greater lethal effect. These results are explained by bypass synthesis that is not associated with molecular recombination; on the contrary, the damage appears to depress recombination at the molecular and genetic levels.

The RAD50 and RAD52 genes are essential in the repair of DNA double-strand breaks in mitotic cells. They are also indispensible in normal meiotic development. Mutations in either gene abolish meiotic recombination; however, it appears that RAD50 acts at an earlier step in meiosis. Rare single-strand interruptions (SSIs) were observed in rad52 strains shortly after the beginning of meiotic DNA synthesis and these appear to be related to recombination. Gentle isolation techniques have allowed the characterization of SSIs as breaks in DNA rather than gaps. Many of the breaks have 3' OH and 5' PO_4 termini. The SSIs do not appear to be randomly distributed based on experiments involving probes for specific chromosomal regions and may correspond to sites or regions involved in normal meiotic recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 60128-04 CGTB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Collaborative Study to Test for "Genetic Drift" in Laboratory Stocks of Ames' Strs.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	M. Shelby	Geneticist	CGTB	NIEHS
	B. Margolin	Mathematical Statistician	BRAP	NIEHS
	K. Risko	Mathematical Statistician	BRAP	NIEHS

COOPERATING UNITS *(if any)*
British Industrial Biological Research Association, United Kingdom

LAB/BRANCH
Cellular and Genetic Toxicology Branch and Biometry and Risk Assessment Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

As part of a 38-laboratory international study individual laboratory stocks of five Salmonella typhimurium tester strains were compared with reference strains in their response to a mutagen, 4-nitroquinoline-N-oxide (4NQO). The results from all laboratories were analyzed in order to determine the levels of agreement within and between laboratories for each Salmonella strain. It was concluded that "genetic drift" of the tester strains was not a significant factor in inter-laboratory variability. Individual laboratory techniques and test conditions appear to be the major sources of laboratory-to-laboratory variability.

GPO 904-917

CHEMICAL PATHOLOGY BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21047-01 CPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Effects of Ochratoxin A Exposure on Bone Marrow Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.A. Boorman

OTHERS:	M.I. Luster	Immunologist	STB	NIEHS
	M.P. Dieter	Physiologist	STB	NIEHS

COOPERATING UNITS *(if any)*

Systemic Toxicology Branch, NIEHS

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ochratoxin A was administered IP to mice over an 8 day period. Histology, bone marrow parameters and macrophage parameters were assayed. Ochratoxin A caused loss of thymic weight, decreased pluripotent stem cells and granulocyte-macrophage progenitors as well as decreased ^{59}Fe uptake in both the marrow and the spleen. These results were published in 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21064-02 CPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bioavailability of TCDD in Missouri Soil

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: E.E. McConnell

OTHERS:	J.A. Moore	Supv. Veterinary Medical Officer	TRTP	NIEHS
	M.W. Harris	Biological Laboratory Technician	TRTP	NIEHS
	J.D. Allen	Biological Laboratory Technician	TRTP	NIEHS
	E. Haskins	Biological Laboratory Technician	TRTP	NIEHS

COOPERATING UNITS *(if any)*

Environmental Protection Agency
Laboratory of Molecular Biophysics, NIEHS

LAB/BRANCH
Chemical Pathology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TCDD (Dioxin) contaminated soil from two sites in Missouri is being investigated to determine the bioavailability in soil. Guinea pigs are being used in the investigation. Results suggest that there is high bioavailability of TCDD in dirt after ingestion. Studies on bioavailability via the skin are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21065-02 CPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Chrysotile Exposure on Bone Marrow Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.A. Boorman

OTHERS:	M.I. Luster	Immunologist	STB	NIEHS
	M.P. Dieter	Physiologist	STB	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Inc., Research Triangle Park, NC 27709

LAB/BRANCH

Chemical Pathology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chrysotile asbestos fibers were administered via inhalation to mice for three days. Bone marrow cellularity, pleuripotent stem cells and macrophage granule progenitors were quantitated for twelve months following exposure. Bone marrow parameters were depressed at all time periods. Ultrastructural examination was used to confirm the deposition of the fibers in the centriacinar region of the lung. These results were published in 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21066-02 CPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Etiology of the Spanish Toxic Oil Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: E.E. McConnell

OTHERS:	J.A. Moore	Supv. Veterinary Medical Officer	TRTP	NIEHS
	M.W. Harris	Biological Laboratory Technician	TRTP	NIEHS
	J.D. Allen	Biological Laboratory Technician	TRTP	NIEHS

COOPERATING UNITS *(if any)*

The Government of Spain
WHO Regional Office for Europe, Copenhagen, Denmark

LAB/BRANCH
Chemical Pathology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Samples of suspect olive/rapeseed oil which were suspected of being part of the Spanish Toxic Oil Syndrome were given to guinea pigs and ducklings. The theory being tested was to rule out the presence of an obscure mycotoxin such as cytochalasin or trichothecene. The suspect oil samples did not produce disease in those laboratory species. Additional oil samples have been received and will be tested. Work is in progress.

PROGRAM RESOURCES BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-21050-01 PRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Microencapsulation As A Means to Administer Chemicals in Feed.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. W. Jameson	Acting Chief	PRB, NIEHS
Others:	T. J. Goehl	Chemist	PRB, NIEHS
	R. L. Melnick	Toxicologist	CTEB, NIEHS
	B. J. Collins	Technician	PRB, NIEHS
	A. Greenwell	Technician	CTEB, NIEHS

COOPERATING UNITS *(if any)*
Carcinogenesis and Toxicology Evaluation Branch

LAB/BRANCH
Program Resources Branch

SECTION
Chemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microencapsulation is a process for completely enveloping tiny masses of solid particles, or liquid droplets in a protective coating which separates the substance from its environment. The use of microencapsulated chemicals for toxicology studies presents a number of advantages, i.e. it permits testing volatile or chemically reactive compounds in the animal diet, minimizes problems with palatability, etc. Volatile and/or reactive chemicals have been encapsulated using a gum/sorbitol matrix and determined to be stable when mixed with rodent feed. Relative bioavailability of the microencapsulated trichloroethylene compared to the neat test material indicates no significant difference in absorption after oral administration. Feeding studies using the microencapsulated trichloroethylene are planned.

SYSTEMIC TOXICOLOGY BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21003-04 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Halogenated Dibenzofurans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	H. B. Matthews	Research Chemist	TRTP	NIEHS
	Yiannakis M. Ioannou	Staff Fellow	TRTP	NIEHS
	Hans Weber	Visiting Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Halogenated dibenzofurans are found worldwide as environmental pollutants. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the degree and position of halogenation. This work has established that 2,3,7,8-tetrachlorodibenzofuran (TCDF), an extremely toxic isomer, is excreted only after metabolism and toxicity is inversely related to metabolic capability. The concept of a threshold body burden for toxicity is currently being tested. The distribution to the fetus will be examined after maternal exposure. The role of body composition on the disposition of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), the most toxic man-made compound known, is being examined in congenic mouse strains which are sensitive or resistant to TCDD toxicity.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21004-04 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Senescent Changes in Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	Michael Dieter	Research Physiologist	TRTP	NIEHS
	William C. Eastin	Research Physiologist	TRTP	NIEHS
	Susan Borghoff	Graduate Student	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.8	0.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Age-related changes in many physiological parameters have long been known to occur. The basis for these alterations is, however, not well understood. Response to various stresses seems to decline with age. Changes in the ability to metabolize exogenous as well as endogenous compounds has been suggested as a cause for altered functions. This work will explore senescent changes in metabolism of several tissues--liver, lung, kidney, small intestine, brain, lymphoid tissues. Enzyme systems such as glucuronyl transferase, β-glucuronidase, and those involved in intermediary metabolism and immune responses will be investigated. Altered distribution and excretion of chemicals in aging animals is being studied in order to elucidate the basis for age-related changes in toxicological responses. Age-related alterations in gastrointestinal absorption are also being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21009-03 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reproductive Effects in Males Exposed to Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James C. Lamb, IV	Research Biologist	STB	NIEHS
Others:	R.E. Chapin	Staff Fellow	STB	NIEHS
	J.K. Dunnick	Biologist	CTEB	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch
Data Management and Analysis
Carcinogenesis and Toxicology Evaluation Branch

LAB/BRANCH

Systemic Toxicology Branch, TRTP

SECTION

Fertility and Reproduction Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	1.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various environmental and industrial chemicals can disturb male reproductive function. The objective of these studies is to enhance our understanding of that toxic potential, and to elucidate the mechanism of action in chemicals found to be toxic. Chemicals which are active as chemosterilants in males, such as glycol ethers, dimethyl methyl phosphonate, dibromochloropropane and the phthalate esters, are used in various test systems. In addition to mechanistic studies, chemicals of unknown activity, such as the phenoxy herbicides and TCDD, have also been studied. Endpoints of toxicity include the assessment of testicular morphology, spermatogenesis, sperm morphology, and hormone levels. Studies continue on the morphological response of the testis to chemical exposure. Androgen Binding Protein (ABP) assays will also be performed to assess Sertoli cell function. Studies are beginning which will evaluate a marker for germ cell response to testicular toxicants. Other studies are beginning to examine any changes of Sertoli cell function in vitro after exposure to toxicants in vivo and in vitro. Cell separation studies will examine biochemical defense mechanisms in isolated germ cell populations after exposure to various toxicants. These studies are expected to yield valuable data on chemical toxicity in males, as well as improve the sensitivity and accuracy of future testing systems.

DHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21024-03 STB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Environmental Chemicals on Drug-Metabolizing Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI.	Joyce A. Goldstein	Pharmacologist	TRTP	NIEHS
Others:	J. Hardwick	Staff Fellow	TRTP	NIEHS
	P. Linko	Chemist	TRTP	NIEHS
	R. Weaver	Biological Lab Tech	TRTP	NIEHS
	P. McClellan-Green	Q-Appointment	TRTP	NIEHS
	H. Yeowell	Visiting Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Biochemical Toxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this study are to examine changes in genetic control of subspecies of cytochrome P-450 in the rat after treatment with polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD) and other environmental chemicals and to assess the implications of these changes. Present work emphasizes effects of PCBs and TCDD on the two major TCDD inducible isozymes ($P\text{-}448_{MC}$ and $P\text{-}448_{MCB}$).

A. AAF Metabolism. The contribution these two isozymes (P-448-MC and P-448-HCB) to metabolism of AAF in control and 3-MC induced microsomes was assessed. P-448-MC contributed chiefly to detoxification (ring hydroxylation) and P-448-HCB to N-hydroxylation (activation) in 3-MC induced microsome.

B. Induction by a PCB Isomer: Dose response curves and time courses indicate that P-448-MC and P-448-HCB are coordinately induced by 3,4,5,3',4',5'-HCB. Induction of mRNA was demonstrated in a cell-free system but the magnitude of induction of the mRNA did not totally account for the increase in protein.

Antibodies are being raised to additional forms of rat liver P450 to be used as probes for quantitating changes due to environmental chemicals. We will attempt to clone DNA complementary to some of the rat P450s to use to analyze gene structure and changes in protein and mRNA synthesis after exposure to various chemicals.

The goal of this project is to better understand the changes occurring after exposure to environmental chemicals. Antibodies and clones developed to these cytochromes will be useful in studying these effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES21025-03 STB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Disposition of Santonox

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS
Others:	H. B. Matthews	Research Chemist	TRTP NIEHS
	W. C. Eastin	Research Physiologist	TRTP NIEHS
	Richard Smith	Visiting Fellow	TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Santonox, 4,4'-thio-bis(6-tert-butyl-m-cresol), has been recommended for study in the NTP as a representative of the class of rubber antioxidants which have widespread industrial usage and a high potential for occupational exposure. Santonox is relatively non-toxic, with an oral LD_{50} of approximately 5g/kg. Before being tested in the bioassay program, disposition studies are needed to assess its absorption, distribution, metabolism, and excretion. Santonox causes severe gastroenteritis after oral exposure. After iv administration, the compound was rapidly conjugated with glucuronicucid in the liver and excreted via the bile into the feces. About 10% of the dose persists in the liver, skin and adipose tissue, suggesting the possibility of accumulation upon chronic exposure.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21026-03 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Hexabromonaphthalene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS
Others:	James D. McKinney	Research Chemist	LEC NIEHS
	Christopher Miller	Guest Worker	TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bromonapthalenes have no known industrial use or application, but have identified as contaminants of Firemaster BP-6, the toxic mixture of polybrominated biphenyls used as a fire retardant and involved in a major episode of environmental posioning in Michigan. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the position of bromination. This work has studied the chemical disposition of a mixture of 2-hexabromonapthalenes (HBNs), previously identified as a single isomer, 1,2,3,4,6,7-HBN. The compound is incompletely absorbed after an oral dose. After iv treatment over 50% of the dose is excreted as metabolites within 3 days. However, the remainder of the dose seems to be extremely persistent, over 25% remaining in the liver after 35 days. These disposition results led to proof of the presence of two isomers by high resolution NMR, present in a ratio of 60:40 which have been tentatively identified as 1,2,3,4,6,7- and 2,3,4,5,6,7-HBN. The difference in the fate of the two isomers has been proven by isolation and characterization of the HBN remaining in the liver 10 days after treatment. While the treatment of HBN was in the isomeric ratio of 60:40 (1,2,3,4,6,7: 2,3,4,5,6,7), the HBN in the liver 10 days after oral treatment was in the ratio of 20:80. The toxicity of this HBN mixture is also being studied. The acute toxic dose response is being defined in mice. This will be followed by an analysis of the teratogenicity of HBN, both alone and in combination with polybrominated biphenyls.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 ES 2 1029-02 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influence of Kepone on Female Reproduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James C. Lamb, IV	Research Biologist	STB	NIEHS
Others:	E.E. McConnell	Veterinary Pathologist	CPB	NIEHS
	K.S. Korach	Research Endocrinologist	LRDT	NIEHS
	J.S. Hong	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch
Laboratory of Reproductive and Developmental Toxicology
Laboratory of Behavioral and Neurologic Toxicology

LAB/BRANCH

Systemic Toxicology Branch, TRTP

SECTION

Fertility and Reproduction Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.5	0.55	0.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of these studies is to evaluate the potential of environmental chemicals to affect female reproductive function. These studies compare toxic effects at high and low levels of exposure. As a model compound for these experiments, we are studying the effects of Kepone on female reproductive function. The toxicity of these compounds is evaluated using a broad spectrum of toxic indicators. Since these effects, and the effects of other environmental compounds, may be mediated through their estrogenic or other hormonal activity, we have established a number of criteria which indicate hormone activity. Uterine, ovarian and pituitary function are studied in morphological and endocrinological studies after Kepone exposure. Pituitary cell responses in vitro are also evaluated. Morphological studies include light and scanning electron microscopy, hormone and xenobiotic autoradiography, and histochemistry. Biochemical studies include hormone radioimmunoassay and hormone receptor assays. These studies will help establish the mechanism of reproductive toxicity of compounds such as Kepone and should lead to more efficient and accurate testing systems in reproductive toxicology.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21036-02 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disposition of Benzo(f)quinoline

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Benzo(f)quinoline (BQ) has been recommended for study in the NTP as a mutagenic air pollutant and as a representative of nitrogen-containing aromatic heterocyclic compounds. It is present in various crude oils. Preliminary studies indicate that it may have carcinogenic potential. Before being tested in the bioassay program, disposition studies are needed to assess its absorption, distribution, metabolism and excretion. BQ was completely absorbed after oral exposure and was rapidly excreted as metabolites in approximately equal amounts into the urine and via the bile into the feces. No radioactivity persisted in the body after acute exposure. Such studies will not only result in more appropriate dose settings for toxicity studies, but a better understanding of the mechanism of toxicity of this compound.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21038-02 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical Metabolism and Disposition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. B. Matthews Research Chemist TRTP NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of chemical metabolism and disposition are designed to provide both applied knowledge in support of chronic toxicity tests conducted by the National Toxicology Program and basic knowledge of those chemical structure and property relationships which determine toxicity. Studies of benzyl acetate indicate that this compound is readily absorbed from the gastrointestinal tract, rapidly metabolized and excreted in urine primarily in the form of hippuric acid. Investigation of parameters of dermal absorption indicate that the effective dose of a chemical which can be applied dermally is limited by the physical properties of that chemical and the vehicle in which it is applied. Studies of the gastric toxicity of a series of acrylate esters indicate that these compounds are strong irritants to the forestomach of the rat, and rats adapt to the irritating effects of these chemicals with chronic exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21042-02 STB

PERIOD COVERED

1 October 1983 through 30 September 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Tumorigenic Potential of Nitrogen Dioxide by Inhalation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.W. Van Stee	Veterinary Officer	TRTP (NTP) NIEHS
OTHERS:	Richard A. Sloane	Biologist	TRTP (NTP) NIEHS
	Jane Ellen Simmons	Graduate Student	TRTP (NTP) NIEHS
	Michael P. Moorman	Engineer	TRTP (NTP) NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Incorporated

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Mice were exposed to approximately 20 ppm of nitrogen dioxide (NO2 or 15-NO2) for 5-6 hours, or 1 g/kg of body weight of morpholine by gavage, or both. Treatments were repeated daily for 5 consecutive days. N-nitrosomorpholine (NMOR) was found in whole carcasses (16 to 146 ng/mouse) in all animals that had been exposed to both NO2 and to morpholine, but not in tissues from animals that had been exposed to either chemical alone. Approximately one-third of the NMOR was found in the gastrointestinal tract. The coadministration of 2g/kg of sodium ascorbate or 1 g/kg of alpha-tocopheryl acetate had no effect on the amount of NMOR that was found in any tissue. Approximately one-third of the total amount of NMOR that was found in the body was found in the stomach. N-nitromorpholine was not detected in any tissue. The results of the 15-NO2 experiments are not yet available. We concluded that the repeated, concurrent exposure of mice to NO2 by inhalation and to morpholine by gavage resulted in the in vivo formation of detectable quantities of NMOR. No tumors were seen in related 2-year studies from which we conclude that concurrent exposure to NO2 and nitrosatable amines probably does not constitute a carcinogenic hazard in laboratory animals.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21043-02 STB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Chemical-Induced Immunotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard W. Pfeifer Senior Staff Fellow STB NIEHS

Others: None

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Immunotoxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ongoing objectives of this project were to develop predictive hypotheses about chemical-induced immunotoxicity. This was achieved with a knowledge of the distribution and metabolism of a compound in conjunction with an understanding of the molecular biology inherent in in vitro assays of immune cell function. Three immunoregulatory circuit models were developed to analyze mechanisms of chemical/immunological interaction. The models included: 1) lectin-induced lymphocyte agglutination (an early event of lymphocyte activation) in conjunction with blastogenesis (a late event); 2) lymphokine-induced macrophage activation (agglutination, cytostasis of growing tumor cells); and 3) lymphocyte-mediated growth inhibition (an effector cell function). This approach identified chemical metabolites with "specific" effects on various components of host defense (ip). Work has been completed with 17-β estradiol and four of its major metabolites including 2-OH estrone (2-OH E), 2-OCH_3 estrone (2-OCH_3 E), estrone (E) and 16α-OH estrone (16α-OH E). Although classical cytosolic receptor binding activity has been demonstrated in lymphoid tissue for estrogens, this work suggested that biological response of target cell populations, in many instances, may be more directly associated with nonspecific membrane effects of these compounds. In particular, an important role for catechol estrogen metabolites was suggested. The models may now be applied to other chemical classes.

PHS 6040 (Rev. 4/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 ES 21046-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Postnatal Toxicology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Douglas J. Kornbrust Senior Staff Fellow STB NIEHS

Others: None

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, TRTP

SECTION

Fertility and Reproduction Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts are in progress to improve our ability to characterize the toxicity of chemicals to neonates relative to adults, and to explore the role of lactation in the induction of neonatal toxicity. The lactational process is evaluated as a source of exposure to chemicals secreted into milk and from the standpoint of nutritional support to the newborn. A pilot study with DDE was performed which, in addition to providing an opportunity to establish and validate various techniques, generated useful information about the capacity of this insecticide to influence various parameters of lactation, including milk production, milk composition (lactose, lipid, protein components), pup growth, and mammary gland integrity (at both the morphological and biochemical levels). Other investigations have been concerned with characterizing the secretion of the liver carcinogen, dimethylnitrosamine, into milk and measuring genetic toxicity in the neonate exposed via the milk.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21069-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Xenobiotics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	L. R. Kao	Visiting Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the disposition of xenobiotics are designed to support the toxicity testing carried out by the National Toxicology Program and to further basic knowledge in the areas of pharmacokinetics and structure/activity relationships. O-benzyl-p-chlorophenol (BCP) is a broad spectrum germicide which is highly irritating and causes renal necrosis. It increases cytochrome P450 in both kidney and liver. It inhibits certain monooxygenase activities. Its absorption, both oral and dermal, tissue distribution, metabolism and excretion will be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21070-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(50 characters or less. Title must fit on one line between the borders.)*

TCDD Teratogenicity: Modulation in Mixtures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	James C. Lamb	Research Bioloigst	TRTP	NIEHS
	James D. McKinney	Research Chemist	LMB	NIEHS
	Martha Harris	Head Technician	TRTP	NIEHS
	Hans Weber	Visiting Fellow		

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.3	0.8	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TCDD (dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin) is one of the most toxic chemicals known to man. Progressive weight loss and thymic atrophy are two of its most frequent toxic symptoms. The induction of cleft palate and hydronephrosis characterize the teratogenic response of mice to TCDD. Because of the sensitivity of this response, we decided to use teratogenicity to measure the interaction of TCDD and other compounds with which it occurs in the environment. Such chemicals include polychlorinated dibenzofurans, polychlorinated biphenyls, hormones such as thyroxine and hydrocortisone, and drugs. The effects on TCDD toxicity are dependent upon chemical structure and may support a mechanistic hypothesis of TCDD toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21071-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Xenobiotic Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Leo T. Burka	Research Chemist	TRTP	NIEHS
Others:	Richard W. Smith	Visiting Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The chemical structures of the major urinary metabolites of three compounds either tested or on test in the NTP have been determined. The major metabolite of allylisothiocyanate in the rat was found to be a mercapturic acid, N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine. The major metabolites of benzyl acetate in the mouse and rat were the glycine conjugate, hippuric acid, and the mercapturic acid, S-benzyl-N-acetyl-L-cysteine. The major metabolites of 2,4-dinitroaniline have been tentatively identified as the sulfate conjugate, N-(2,4-dinitrophenyl) hydroxylamine-O-sulfonic acid and the corresponding hydroxylamine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21072-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methyl Carbamate: Comparative Disposition in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yiannakis M. Ioannou Senior Staff Fellow TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methyl carbamate (MC) has been recently studied by the National Toxicology Program for chronic toxicity and carcinogenicity. MC was found to be toxic to rats but had no effect on mice. The present study was designed to investigate mechanisms of action which can account for the observed species specificity in MC toxicity. Results of these studies indicate that although MC is readily absorbed and rapidly distributed to all tissues, in both rats and mice, mice are much more efficient in clearing MC and/or metabolites from the tissues than rats. Within 72 hours after administration greater than 95% of the dose is eliminated by mice while in rats over 50% of the dose is still present in the tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21073-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of 1,2-Dihydro-2,2,4-trimethylquinoline in Male F344 Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yiannakis M. Ioannou Senior Staff Fellow TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The absorption, distribution, metabolism and excretion of ^{14}C-labeled 1,2-dihydro-2,2,4-trimethylquinoline (TMQ) was studied in male F344 rats. TMQ was readily absorbed, distributed to all tissues, metabolized and excreted mainly in the urine in the form of several metabolites. The rate of clearance from the tissues is rapid and the whole body half-life is less than 12 hours. Only liver retained significant quantities of TMQ-derived radioactivity 24 hours after an intravenous dose of 115 μmole/kg body weight. In vitro studies indicated that TMQ is readily metabolized by hepatic mixed-function oxidases and that some of the initial metabolites are conjugated prior to excretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21075-01 STB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leo T. Burka Research Chemist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	0.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome P-450-containing mixed function oxidase is one of the major enzyme systems responsible for metabolism of xenobiotics. Chemical mechanisms for cytochrome P-450 metabolism have been investigated using correlation of Vmax and Km to Hammett's sigma parameter and Hansch's hydrophobic pi parameter. It has been found that the difference in regiospecificity in hydroxylation of monohalobenzenes by phenobarbital and beta-naphthoflavone induced microsomes is due to a difference in chemical mechanism. The electronic demand for the rate determining step for N- and O-dealkylations has been investigated using the same correlations and evidence has been obtained that supports rate limiting one electron oxidation of the amine nitrogen to the radical cation. A corresponding one election oxidation of the ether oxygen apparently does not occur.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES30044-08 STB

PERIOD COVERED

1 October 1983 through 30 September 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toxicology of Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.W. Van Stee	Veterinary Officer	TRTP (NTP) NIEHS
OTHERS:	Richard A. Sloane	Biologist	TRTP (NTP) NIEHS
	Jane Ellen Simmons	Graduate Student	TRTP (NTP) NIEHS
	Michael P. Moorman	Engineer	TRTP (NTP) NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Incorporated

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thyroid function in rats and rabbits was measured following inhalation exposure to carbon disulfide. Serum T4 activity decreased in rabbits but not in rats. Other indexes of thyroid function remained unchanged. Rabbits were exposed or treated with all combinations of carbon disulfide, cholesterol-enriched diet, thiourea, and levothyroxine. Histopathological examination did not support the hypothesis that in the rabbit carbon disulfide accelerated the atherosclerotic process through depression of thyroid activity. Other studies in rats demonstrated that exposure to carbon disulfide reversibly alters hepatic cholesterol metabolism through a mechanism that is dependent on the prior metabolism of carbon disulfide. Exposure of strain A mice for 6 months to vinyl chloride, ethylene dibromide or ethylene oxide, but not to carbon disulfide or NO2+morpholine cause concentration-related increases in numbers of pulmonary adenomas that were formed. This inexpensive model may be useful in helping to identify inhalant carcinogens.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30106-10 STB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
The Effects of Environmental Pollutants on the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Michael I. Luster	Research Microbiologist	STB	NIEHS
Others:	G. Boorman	Veterinary Medical Officer	STB	NIEHS
	A. Tucker	IPA, Medical College of Virginia	STB	NIEHS
	K. Korach	Research Chemist	LPRT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Immunotoxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER.
4.25	1.5	2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ongoing objectives of the immunological-toxicology group include the following interrelated efforts: (1) to evaluate and examine the influence of selected environmental chemicals on the immune response including cellular changes associated with chemical interactions in lymphoreticular cells; (2) to relate alterations in immunologicl functions with both general toxicity as well as specific organ toxicity; (3) to relate changes in immunological functions with altered host resistance following challenge with either syngeneic tumor cells or infectious agents employing a defined panel of infectivity models; and (4) to refine and validate a panel of immune and host resistance procedures in order to better define immunotoxicity and correlate changes in immune function with altered host resistance. This approach should potentially allow for more accurate assessment of human health risk as well as determine no-effect levels for immunotoxic chemicals.

GPO 904-917

BIOMETRY AND RISK ASSESSMENT PROGRAM

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 43009-01 BRAP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Kidney & Nutritional Factors in Metabolism of Toxic & Essential Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert A. Goyer	Deputy Director	NIEHS
Others:	Winona Victery	Expert	EB, BRAP, NIEHS
	Chris R. Miller	Bio. Lab. Techn.	EB, BRAP, NIEHS
	Shiya Zhu	Visiting Fellow	EB, BRAP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of the Director, Biometry and Risk Assessment Program

SECTION
Applied Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Renal function and morphology are studied in rats following exposure to toxic metal, lead or cadmium. Trace mineral excretion is measured before, during, and after therapeutic chelation. Effects of metal toxicity on renal handling of glucose are also being investigated. Parameters being measured include glucose reabsorptive capacity, glomerular filtration rate, and renal plasma flow in animals of different ages with and without toxic metal exposure. Dose-response is being studied using purified diets with defined essential metal content.

Results to date indicate that lead exposure increases urinary excretion of calcium, zinc, copper, iron, and magnesium. Treatment of control and lead exposed rats with the calcium salt of EDTA produces further increased excretion of lead, zinc, copper and iron. Increased calcium excretion does not exceed the content of administered calcium chelate. Animals fed diets containing excess essential minerals and exposed to 1000 ppm lead in drinking water for periods up to 14 months (blood lead <25 µg/dl) showed no differences in maximum glucose reabsorption, glomerular filtration rate, or renal plasma flow. After seven to ten weeks of drinking water with 10,000 ppm lead (blood lead @ 40 µg/dl), animals showed a small increase in glucose reabsorption rate; this appears to be directly related to an increase in kidney weight.

The purpose of these studies is twofold; one is to determine the effects of specific toxic metals on renal function. The second is to determine the influence of altering dietary content of essential metals on parameters of renal toxicity and to establish dose-response relationships.

EPIDEMIOLOGY BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43001-12 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Demographic Investigations of Potential Human Health Hazards

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Walter J. Rogan	Medical Officer	EB	NIEHS
Clarice Weinberg	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

Statistics and Biomathematics Branch, NIEHS; Environmental Epidemiology Branch, NCI; Department of Medicine, University of North Carolina

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	.30	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of vital statistics, demographic and other population data often leads to important clues linking environmental exposures to specific diseases. The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of demographic investigations. The influence of environmental and occupational factors on mortality from chronic renal failure continues to be explored using mortality data for U.S. counties, census data, and data from the NIOSH occupational hazards survey. Data from the Health and Nutrition Examination Survey are being used to develop a new estimate of the number of persons who have been exposed to asbestos and to relate x-ray abnormalities to other measures of lung function. Data from a large British study of sudden infant death are being exploited to develop a measure of sinus arrhythmia in newborns. Existing data, including vital statistics, are also being used to make estimates of the clinical significance of findings from other epidemiologic studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43002-08 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Breast Milk and Formula Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter J. Rogan	Medical Officer	EB	NIEHS
Beth C. Gladen	Statistician	SBB	NIEHS
James D. McKinney	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)* Statistics and Biomathematics Branch, Laboratory of Molecular Biophysics, NIEHS; Raltech, Inc., Madison, WI; Science Applications, La Jolla, CA; Wake Area Health Education Center, Raleigh, NC; Durham Women's Clinic, Durham, NC; East Carolina School of Medicine, Greenville, NC

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Widespread contamination of human milk by polychlorinated biphenyls (PCBs) and 1,1-(p-chlorobiphenyl)-2,2-dichloroethane (DDE, a metabolite of the pesticide DDT) is well documented, but illnesses resulting from such exposure to nurslings are essentially unstudied. This project involves: (1) development of sampling and PCB/DDE analysis methods for breast milk and other tissues and fluids that are reliable, reproducible, and contaminant free; (2) establishment and follow-up of a cohort of children for whom analyzed samples of milk and clinical data are available; (3) development of alternate methods of chemical analysis that are faster or cheaper than gas liquid chromatography; (4) evaluation of the children for specific outcomes thought to be related to DDE/PCB exposure; (5) data cleanup, editing and analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43004-06 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Environmental Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Richard B. Everson	Medical Officer	EB	NIEHS
Walter J. Rogan	Medical Officer	EB	NIEHS
Allen J. Wilcox	Medical Officer	EB	NIEHS
Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

Bowman Gray School of Medicine/Baptist Hospital; Duke University Medical Center; University of North Carolina Medical School; Charlotte Memorial Hospital; Food and Drug Administration, Center for Disease Control

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There are a number of chronic diseases with poorly understood etiologies that contribute substantially to the morbidity of human populations and result in significant expenditures of public health dollars. Environmental agents may produce some of this disease, and identification of associations between exposures and certain diseases would presumably lead to the prevention of morbidity. Other than cancer, few chronic diseases have received much attention in studies of environmental hazards, yet other diseases might be the more common results of exposure to such hazards. The Epidemiology Branch is developing a program in environmental epidemiology that will address the role of environmental factors in the etiology of some less well studied chronic diseases. Studies of risk factors for cancer are also conducted when cancer is the most likely outcome of exposure or when methods for the study of cancer risk are more readily available. The program is also concerned with the development of methods for assessing environmental exposures in the context of epidemiologic studies and examining disease risk in relation to some of these measures of exposure. A multi-center case-control study of risk factors for chronic renal failure is currently being conducted. Related studies involve the development of a renal disease classification scheme for use in etiologic studies and the identification of risk factors for particular renal disease subtypes. The feasibility of other studies that relate to risk factors for chronic renal failure is also being explored. A study of the relationship of childhood and/or adulthood passive exposure to cigarette smoke and cancer risk has been completed and related proposals are being developed. Studies of the etiology of Reye's Syndrome and the specificity of the liver pathology seen in Reye's have also been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43008-05 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Biochemical and Cellular Environmental Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard B. Everson	Medical Officer	EB	NIEHS
Karsten Lundgren	Visiting Fellow	BAS	NIEHS
Thomas K. Wong	Staff Fellow	BAS	NIEHS

COOPERATING UNITS *(if any)* Columbia University; U.S. Department of Agriculture Western Regional Research Center, University of North Carolina at Chapel Hill, Duke University, Baylor College of Medicine

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the effective use of biochemical and cellular assays of human tissue and body fluid specimens in epidemiologic studies seeking evidence of adverse effects associated with specific environmental exposures. It emphasizes interdisciplinary development of ideas and methodologies coupled with attention to details of both the laboratory procedures and the gathering and analysis of data concerning human subjects. Current effort focuses on the development of techniques for identifying genetic damage and alterations in metabolism associated with human exposure to potentially toxic substances. These techniques are being used in model studies of individuals exposed to known amounts of carcinogenic and mutagenic agents used for cancer chemotherapy, occupational groups, smokers, and individuals accidentally exposed to large quantities of PCBs. These studies are designed to help evaluate and refine assay and clinical methods, to investigate mechanisms involved in specific models of human disease, and to investigate the effects of exposures that may be important to public health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 44003-07 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen J. Wilcox	Acting Chief	EB	NIEHS
Beth C. Gladen	Statistician	SBB	NIEHS
Clarice R. Weinberg	Statistician	SBB	NIEHS
Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Carl A. Keller	Epidemiologist	EB	NIEHS
Bruce C. Nisula	Medical Officer	DEB	NICHD

COOPERATING UNITS *(if any)* Developmental Endocrinology Branch, Epidemiology Branch, National Institute of Child Health and Human Development; Department of Medicine, College of Physicians and Surgeons, Columbia University; Department of Medicine, Louisiana State University Medical Center

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.30	1.30	2.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reproductive epidemiology program emphasizes the development and application of new methods for measuring and analyzing human reproductive outcomes. Such outcomes include fertility, sub-clinical early fetal loss, spontaneous abortion, fetal growth, and birthweight. Each of these outcomes can be affected by environmental factors, and represents a possible endpoint for studying the effects of toxins on human reproduction. One major component of this program is a prospective study of early fetal loss among 200 women. Daily urine specimens are being collected from women who have discontinued their use of birth control in order to become pregnant. Urine assays for human chorionic gonadotropin are being used to estimate the risk of early pregnancy loss among these women. A pilot study of the first thirty women enrolled found chemical evidence of four sub-clinical pregnancy losses. Risk of early loss will be studied in relation to common exposures in this population, such as use of alcohol, tobacco, caffeine beverages and medications. Another area of interest is the possible usefulness of measuring fertility through retrospective estimates of time to pregnancy. A pilot study using this approach has recently been completed. Another pilot study is in progress that looks for sub-clinical conceptions among women using IUD's as possible measure of human fertility. Work continues on the further development of a new method for the analysis of birth weight.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 46001-01 EB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptor Action and Liver Tumor Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	George W. Lucier	Chief	BAS	NIEHS
Others:	C. Mastri	Visiting Fellow	BAS	NIEHS
	A. Vickers	NIH Postdoctoral	BAS	NIEHS
	T. Sloop	Biologist	BAS	NIEHS
	Z. McCoy	Bio. Lab. Tech.	BAS	NIEHS
	D. Campen	Research Biologist	BAS	NIEHS

COOPERATING UNITS *(if any)*

Pathology Branch, NTP
Lab of Molecular Biophysics, IRP

LAB/BRANCH

Epidemiology

SECTION

Biochemical Application

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is the long range plan of this project to evaluate actions of receptors for toxic halogenated aromatics and estrogenically-active chemicals in relation to hepatotoxic potency of these compounds. These studies focus on receptor mediated effects on gene expression critical to tumor promotion using the rat two-stage model. The compounds of special interest are 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), structurally-related chlorinated dibenzodioxins and dibenzofurans, diethylstilbestrol, ethinylestradiol and α-zearalanol. The goals of these studies are to quantify the amount of occupied receptor (chronic exposure) necessary to produce a specified risk of liver tumors in rats that had been treated previously with an initiating agent.

STATISTICS AND BIOMATHEMATICS BRANCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40004-07 SBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods in Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Beth C. Gladen	Statistician	SBB NIEHS
Clarice Weinberg	Mathematical Statistician	SBB NIEHS
Takashi Yanagawa	Visiting Associate	SBB NIEHS

COOPERATING UNITS *(if any)*

Department of Mathematics, Kyushu University, Japan (Yoshihiko Maesono)
Department of Epidemiology and Health, McGill University (Sholom Wacholder)

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's activities in the field of epidemiology. The objectives are both to broaden understanding of the uses and limitations of currently employed study designs and corresponding analyses; and to develop new techniques for statistical analyses of epidemiological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40005-07 SBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methodology and Analysis of Mutagenesis Testing Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Ken Risko	Mathematical Statistician	SBB	NIEHS
Doug Simpson	Mathematical Statistician	SBB	NIEHS
Randy Tobias	Mathematical Statistician	SBB	NIEHS
Errol Zeiger	Head, EMTDP	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Cellular Genetics and Toxicology Branch, TRTP

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of appropriate statistical techniques for the analysis of mutagenesis data arising from short-term assays under study by NTP remains the long-term objective of this ongoing project. Statistical procedures for the design and analysis of short-term tests proposed or currently employed by other researchers in mutagenicity are assessed and new and improved procedures are developed. Methodology for the combination of results from a series of experiments in one laboratory, or from a set of experiments conducted by different laboratories is increasingly of interest. Results obtained are applicable to large mutagenicity studies, such as the International Collaborative Study on 'Genetic Drift' in Salmonella Typhimurium Strains and the Collaborative Study on Short-Term Tests conducted by the International Program for Chemical Safety, WHO. The major focus to date has been on in vitro microbial test systems, although additional research has dealt with tests employing Drosophila and mammalian cells in culture. Future efforts will expand shortly to in vivo test systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 41001-10 SBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Risk Assessment Methodology Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David G. Hoel	Chief	SBB	NIEHS
Michael D. Hogan	Mathematical Statistician	SBB	NIEHS
Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Nathaniel B. White	Mathematical Statistician	SBB	NIEHS
Eiji Yamamoto	Visiting Fellow	SBB	NIEHS
Marshall W. Anderson	Mathematician	BAS	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Developmental Toxicology; Developmental Biology Division, Health Effects Research Laboratory, EPA

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the development of statistical/mathematical methodology useful in the assessment of human health risks associated with exposures to potentially hazardous environmental agents. The primary focus is on the generation of improved statistical techniques for estimating adverse human health effects from laboratory animal data; and special emphasis is placed on dose-response modeling, low-dose extrapolation and extrapolation of toxicologic responses across species. Consideration is also given to the modeling of epidemiologic data in the risk assessment process. Present research efforts are concerned with the distribution of virtually safe dose level estimates under a two-stage model of carcinogenesis, with the estimation of working lifetime effects from continuous low-level exposures based on short-term occupational exposure data, with the impact of pharmacokinetic considerations on dose-response modeling in carcinogenesis, and with various methodological issues bearing on teratogenic risk assessment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 44002-09 SBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mathematical Modeling of Molecular Phenomena

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Charles H. Langley	Research Chemist	LAG	NIEHS
Tom Darden	Staff Fellow	SBB	NIEHS
Dick Hudson	Staff Fellow	SBB	NIEHS
Michael Resnick	Geneticist	TRTP	NIEHS
Charles Aquadro	Staff Fellow	LAG	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Animal Genetics, LRDT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall objective of this research project is the development of mathematical models to describe certain biological phenomena at the molecular level. Current efforts include the development of stochastic models to describe the evolution of transposable elements in finite Mendelian populations, the continued investigation of models used to predict nucleotide substitution rates from restriction enzyme data and nucleotide sequence data, the development of stochastic models which describe the interactions of genetic recombination with DNA repair and normal meiosis at the molecular level, and the development of efficient Monte Carlo methods to study the statistical properties of samples from populations evolving according to the neutral model. Work has also been initiated in the area of computational chemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 45001-04 SBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Design and Data Analysis Methodology for Animal Experiments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
David G. Hoel	Chief	SBB	NIEHS
Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Gregg E. Dinse	Staff Fellow	SBB	NIEHS
Takashi Yanagawa	Visiting Scientist	SBB	NIEHS
Gary T. Brooks	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with statistical methodology issues involved in the design and analysis of animal experiments with particular emphasis on TRTP's two-year carcinogenesis studies. Specific research areas include the utilization of historical control data, examination of Type I error rates and the development of statistical methodology to facilitate data analysis of diseases with uncertain cause of death.

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